BOSTON UNIVERSITY

GRADUATE SCHOOL

Dissertation

THE DETERMINATION OF SODIUM IN BIOLOGICAL FLUIDS

by

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submitted in partial fulfilment of the

requirements for the degree of

Doctor of Philosophy

1939

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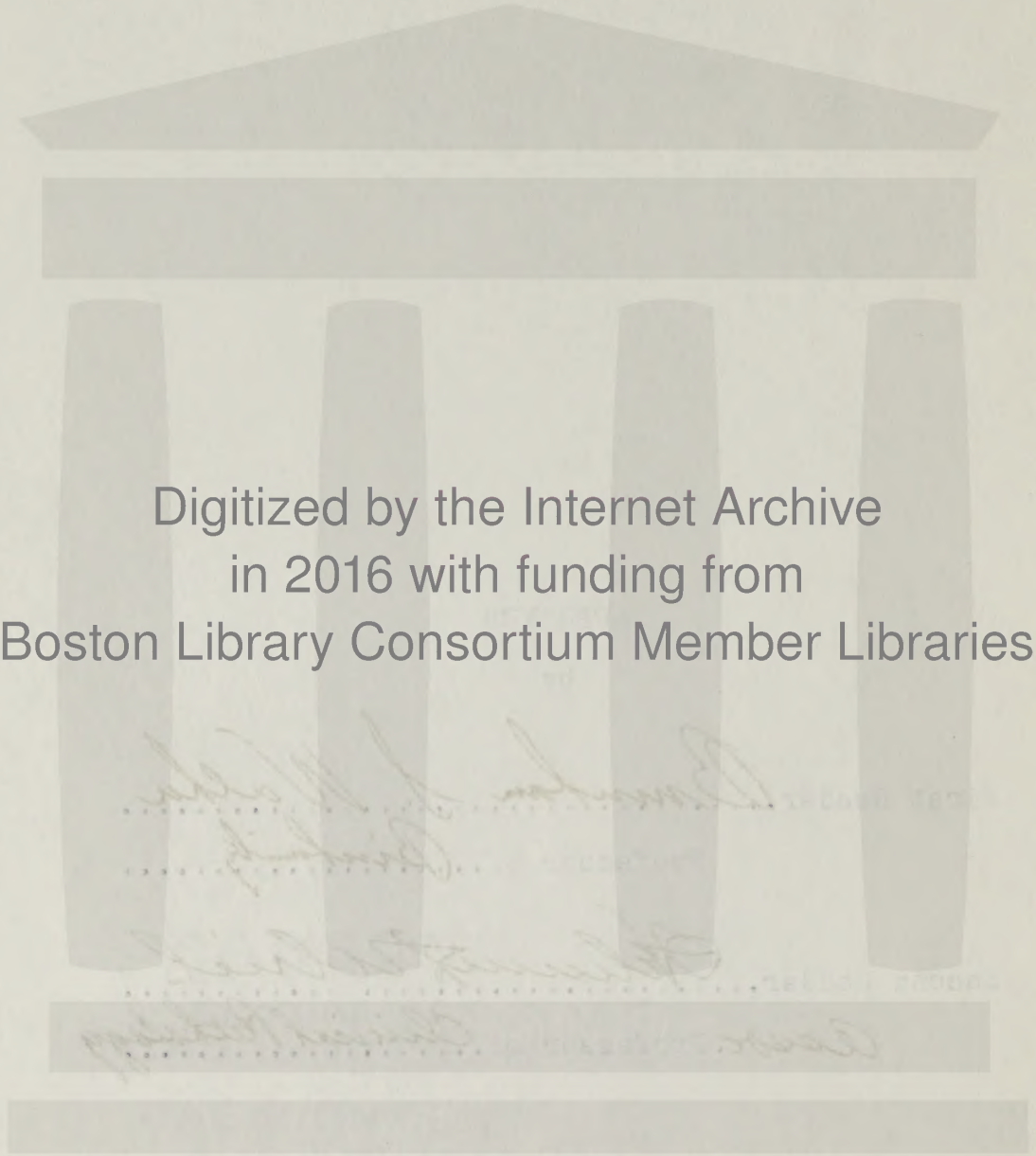

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Introduction

Recent work on sodium metabolism in adrenal insufficiency, disturbed acid-base equilibrium, and forced loss of fluids (57,58) has changed the status of the determination of sodium in biological fluids from a test rarely done in the clinical laboratory to one that is rapidly becoming necessary in the armamentarium of the average clinical laboratory technician. Although cases have been reported in which twenty-five percent or more of the sodium of the body has been lost (59), the body generally maintains homeostasis with respect to sodium to such an extent that deviations in the sodium content of body fluids of four percent from the mean normal figure may be presumed to be indicative of a beginning pathological process (36). Thus, to be of any value, a method of determination of sodium must be accurate to one percent. This contribution represents an attempt to develop a method, with a maximum error of one percent, which shall be simple enough to be performed in routine clinical laboratories.

Review of Literature

Methods not employing uranyl triple salts

The classical method for the determination of sodium requires the removal of all ions but sodium and potassium, the precipitation of sodium and potassium as chlorides, the separation of potassium as chloroplatinate or perchlorate, and the calculation of sodium by difference (72, 94). McLean and Van Slyke (62), in 1915, devised a method for titration of chloride by adding an excess of silver nitrate and titrating the excess with potassium iodide. McCrudden and Sargent (61) applied this titration to the determination of sodium; by precipitating both sodium and potassium as chlorides and determining the chloride content of the weighed precipitate, they calculated the percentage of sodium in the precipitate. These authors pointed out that any error in technic is magnified in calculation.

Fenton (28), in 1898, proposed a method for the determination of sodium, based on the insolubility of the sodium salt of dihydroxytartaric acid in cold water. This method has not been used to any great extent.

Ball (5) introduced a gravimetric method precipitating sodium as the complex bismuth caesium nitrite having the composition $9CsNO_2 \cdot 6NaNO_2 \cdot 5 Bi(NO_2)_3$. This method was modified and improved by Doisy and Bell (22), who determined the nitrite content of the precipitate volumetrically and colorimetrically.

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Kramer (47) precipitated sodium as the pyroantimonate $Na_2H_2Sb_2O_7 . 6H_2O$ in alcoholic medium, and weighed the precipitate. This gravimetric method was used by Kramer and Tisdall in several studies (49, 50, 92) of sodium in biological fluids. In 1924 Bálint (3) and, independently, Kramer and Gittleman (48) adapted the pyroantimonate precipitation to a volumetric procedure by adding potassium iodide to the dissolved precipitate and titrating the liberated iodine. The method was refined by Kerr (42) and by Rourke (79), who removed protein from the serum or plasma before adding the pyroantimonate reagent. This iodometric method has been applied to milk by Sato and Murata (84). Lewin (53) dissolved the precipitated pyroantimonate in HCl, reduced the antimony with sodium sulfite, boiled off the excess of sulfur dioxide, and titrated with standard bromate solution. A colorimetric method, based on the measurement of the orange color of antimony trisulfide obtained from the pyroantimonate precipitate, was devised by Yoshimatsu (98).

Stoddard (89) precipitated out the alkaline earths, electrolyzed the remaining solution in two connected tubes of mercury, dissolved the sodium and potassium out of the resulting cathode amalgam, and titrated with standard acid. The value of potassium, determined by the method of Fiske and Litarczek (29), when subtracted from the value of sodium plus potassium obtained from the titration, gave the figure for sodium.

Brown and Shohl (10) determined total base in blood by the method of Stadie and Ross (88); then by quantitation of other cations, they obtained a figure for sodium plus potassium.

Thomson and Lee (91) adapted spectrographic analysis, as developed by Russanov(80), Jansen, Heyes, and Richter (38), and Duffendack, Wolfe, and Smith(23), to biological fluids.

Prinsen-Geerlings (76) in 1937 published an article on the determination of serum sodium by the polarograph, an instrument which registers current voltage lines automatically; no figures were reported in the paper, so no evaluation of the method is possible.

Methods employing uranyl triple salts

Since 1886, when Streng (90) isolated and identified a triple acetate of uranium, magnesium, and sodium, a series of similar uranyl-sodium-divalent metal acetates has been prepared. They possess the common characteristics of easy solubility in water and sparing solubility in alcohol. Kolthoff (45) prepared the zinc salt $(UO_2)_3ZnNa(CH_3COO)_9 \cdot 6H_2O$ and used its formation as a qualitative test for sodium. Caley (13) prepared the corresponding cobalt salt and used cobalt uranyl acetate as a qualitative test for sodium. The eleventh United States Pharmacopoeia (73) also has adopted this test. Feldstein and Ward (27) used nickel uranyl acetate as a qualitative reagent for sodium. Chang

and Tseng (18) have used the manganous salt in quantitative determinations. There is concurrence among the users of these triple salts that the cobalt, manganese, zinc, and nickel salts are isomorphic, having the formula $(UO_2)_3$ Na-M^{++} $(CH_3COO)_9$.$6H_2O$, where M^{++} represents the divalent metal. There is strong disagreement over the composition of the magnesium salt. Streng (90) and Blanchetière (9) stated its composition as $(UO_2)_3MgNa(CH_3COO)_9.9H_2O$; Caley and Foulk (17) assigned 6.5 molecules of water of crystallization to it; Nydahl (66), Kahane (40), and Raszeja(77, 78) 8 molecules of water; and Miholic (63), Barber and Kolthoff (6), and Alten, Wieland, and Hille (2), 6 molecules.

After one of the triple salts has been separated from the solution, the quantitation may proceed gravimetrically, volumetrically, or colorimetrically.

All gravimetric methods involve the washing, drying, and weighing of the isolated triple salt. The precipitate is washed first with alcohol (6) or glacial acetic acid (82) saturated with the triple salt, then with ether. The manganese and zinc salts may be dried in air, without the use of desiccators or ovens. The uncertainty of composition of the magnesium salt makes it necessary for each person using it to adhere to his own set of standard conditions for maintaining constant composition of the precipitates.

The gravimetric method using the magnesium triple salt, as developed by Streng (90), Miholic (63), Blanchetière (9),

Caley and Foulk ([14][17]), Kahane (40), and Kolger (44), has been found inaccurate by Kling and Lassieur (43) and by Barber and Kolthoff (6). Caley (16) has improved the method and limited the errors to about ± 1.5 percent.

Barber and Kolthoff (6) developed a gravimetric method based on the weighing of the zinc triple salt, and demonstrated the stability of composition and ease of washing and drying of the precipitate. Later (7) they showed that the chief interfering substances are potassium, phosphate, and arsenate. Butler and Tuthill (12) applied the method to biological fluids and obtained excellent results after destruction of proteins. Jendrassik and Dziobek (39) adapted the method to the analysis of minute quantities of biological fluids by use of a sensitive torsion balance.

Chang and Tseng (18) found that uranyl manganese acetate is as satisfactory a reagent for the gravimetric determination of sodium as is uranyl zinc acetate.

The first volumetric determination of sodium based on triple salt separation was devised in 1930 by Caley (15), who dissolved the precipitate of uranyl magnesium sodium acetate and titrated the uranium with a standard phosphate solution till paper impregnated with potassium ferrocyanide showed no red color when dipped in the titration mixture. Rusznyák and Hatz (81) devised a similar procedure for the zinc triple salt. They added an excess of phosphate to precipitate the zinc and the uranium and titrated the excess phosphate with standard uranyl acetate solution.

In the volumetric method of Dobbins and Byrd (21) an excess of sodium hydroxide is added, converting the uranium and zinc in the zinc triple salt to the amphoteric hydroxides, according to the equations

1. $3UO_2^{++} + Zn^{++} + Na^+ + 9CH_3COO^- + 8Na^+ + 8OH^- \longrightarrow$

$$3UO_2(OH)_2 + Zn(OH)_2 + 9Na^+ + 9CH_3COO^-$$

2. $3UO_2(OH)_2 + Zn(OH)_2 + 8\ Na^+ + 8OH^- \longrightarrow$

$$8Na^+ + 3UO_4^{=} + ZnO_2^{=} + 8H_2O\ .$$

By titrating the excess sodium hydroxide with standard acid to a phenolphthalein end point, reaction 2 is entirely inhibited and a stoichiometric relationship exists between the amount of sodium hydroxide consumed and the amount of triple salt present. Sassier (83) used this method for the determination of sodium in urine. Weinbach (96), in applying the method to biological fluids, titrated the zinc and uranium directly with sodium hydroxide to a phenolphthalein end point.

It has long been known that uranyl compounds can be reduced from the hexavalent to the tri-and tetravalent states in acid solution, and that any trivalent uranium can be oxidized to the tetravalent state by aeration (52). This reaction was applied to the zinc triple salt by Gall and Heinig (31), who reduced with electrolytic cadmium, aerated, and titrated the tetravalent uranium with permanganate. Chen (19) used a similar procedure. Furman, Caley, and Schoonover (30) precipitated sodium as the magnesium triple salt, reduced the uranium by passing the acid solution

In the volumetric method of Dobbins and Byrd (21) an excess of sodium hydroxide is added, converting the uranium and zinc in the zinc triple salt to the amphoteric hydroxides, according to the equations

1. $2UO_2^{++} + Zn^{++} + Na^+ + 6OAc^- + 8NaOH \longrightarrow$

$2UO_2(OH)_2 + Zn(OH)_2 + 2Na^+ + 6CH_3COO^- + 8Na^+ + 8OH^-$

2. $2UO_2(OH)_2 + Zn(OH)_2 + 6\ Na^+ + 8OH^- \longrightarrow$

$8Na^+ + 2UO_2^= + ZnO_2^= + 8H_2O$.

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through a Jones reductor, aerated, added an excess of ceric sulfate, and back-titrated with ferrous sulfate. Kahane (40) precipitated the magnesium salt, dissolved in acid, reduced with aluminum, and titrated directly with permanganate.

Kano (41) introduced the use of amalgams as reducing agents for uranium determination. Someya (87) used bismuth amalgam for the determination of uranium. He worked out a method for sodium determination in which he precipitated with uranyl magnesium acetate, dissolved the precipitate in acid, reduced with bismuth amalgam, aerated, and titrated with potassium dichromate. Kolthoff and Lingane (46) used a similar method, employing the zinc triple salt, zinc amalgam, and dichromate. Raszeja (78) precipitated the sodium of blood as the magnesium triple salt, reduced with zinc amalgam, and titrated with permanganate. Ball and Sadusk (4) used the Kolthoff and Lingane method for determining blood sodium, and Holmes and Kirk (37) used cadmium amalgam as the reductant and ceric sulfate as the oxidant.

Volumetric procedures have also been worked out for the determination of zinc in zinc uranyl sodium acetate (51) and for acetate in magnesium uranyl sodium acetate (24).

The first reliable colorimetric test for the detection of uranyl compounds was published by Bruttini (11) in 1893. It depends on the formation of a reddish brown uranyl potassium ferrocyanide when potassium ferrocyanide is added to an acid solution of a uranyl salt. In 1927, after the application

of the uranyl magnesium acetate reagent to the determination of sodium by Blanchetière (9), Barrenscheen and Messiner (8) developed a colorimetric method for the determination of blood sodium based on the formation of the ferrocyanide of the uranium in the magnesium triple salt. Poulsson (74) and Tissier and Bénard (93) used similar methods. McCance and Shipp (60) used the ferrocyanide color from the zinc triple salt as a measure of blood sodium. Their method was adapted to other biological fluids by Salit (82), Alten and Wieland (1), Marenzi and Gerschmann (55, 56) Malyarov and Yudenich (54), and Oberst (67).

Caley and Foulk (17), at the outset of their work with uranyl magnesium sodium acetate, appreciated the possibility of measuring the intensity of the yellow color of solutions of the salt as a quantitative procedure. Hoffman and Osgood (36), using uranyl zinc sodium acetate, measured the light absorption in a photoelectric colorimeter (85) after stabilizing the color with ammonium thiocyanate.

Müller (65), in 1919, discovered that sodium salts of alpha-hydroxy and alpha-keto acids and of aromatic hydroxy carboxylic acids give yellow or red colors with uranyl salts in the absence of free mineral acid. Elías (25) developed a colorimetric micromethod for determining sodium by dissolving the precipitate of magnesium uranyl sodium acetate and developing the color with sodium salicylate.

Das Gupta (20) found that phenolic acids generally give

colors with uranyl salts and that sodium acetate intensifies the colors. He recommended gallic and tannic acids as colorimetric reagents for uranium.

Ogden (68) used cochineal as an external indicator for excessive uranium in the titration of phosphate with uranyl acetate. Germuth and Mitchell (32) propose sodium alizarin sulfonate as a color reagent for uranium.

Woelfel (97) has recently devised a colorimetric method for the determination of sodium, based on precipitation with uranyl manganese acetate, oxidation of the manganese to permanganate, and measuring the color.

Experimental

Choice of Method

Since the introduction of the photoelectric colorimeter into biochemical laboratories (26,85), the speed and ease of operation of the instrument and the minimum of subjective errors entailed in its use have made the photoelectric measurement of color very popular. The simplification of any method of analysis without the sacrifice of accuracy is the goal of all analysts. By a simple survey of the limits of accuracy of the Evelyn (26) photoelectric colorimeter, it can be shown that the error involved in the use of the instrument can be held to .75%.

Getman and Daniels (33) state Beer's law as follows:

$$I = I_o e^{-k'lc}$$

where

I_o is the intensity of light striking the absorbing medium,

I is the intensity of light after having passed through a depth l of the medium,

c is the concentration of the absorbing medium,

e is the base of natural logarithms, and

k' is a constant characteristic of the absorbing material and of the wave length of the light passing through.

In the Evelyn colorimeter the measurements of absorption are always made through standard tubes, so that l is likewise constant. The tube containing the colored material is placed between a lamp and a photocell, so that the transmitted light

falls on the photocell. The cell is connected to a galvanometer, the scale of which is graduated into one hundred equal parts; the maximum error of reading is one quarter of a scale division. Before making the determination, the galvanometer is adjusted to read 100 when a tube containing pure solvent (reaction mixture minus the substance to be determined; that is, zero concentration of the colored substance) is placed between the lamp and the photocell. Then, when the tube containing the colored substance is placed between the lamp and the cell, the light transmitted, as measured in percentage of that transmitted by the blank tube, is given directly by the galvanometer reading. The transmission of the sample solution, defined as the ratio of the light transmitted by the sample to that transmitted by the blank, is given by the expression

$$T = \frac{G}{100},$$

in which T is the transmission and G is the galvanometer reading of the sample solution.

Since the concentration of colored substance in the blank tube is zero, then by Beer's law the intensity of light transmitted by the blank tube

$$I_b = I_o e^{-k'0}$$

or $$I_b = I_o e^{0}.$$

Since $e^{0} = 1$,

$$I_b = I_o.$$

Now considering the intensity of light transmitted by the sample, $I_x = I_o e^{-k'lc}$.

The ratio of light transmitted by sample and blank

$$T = \frac{I_x}{I_b} = \frac{I_o e^{-k'lc}}{I_o} = e^{-k'lc}.$$

And since $T = \frac{G}{100}$,

$$\frac{G}{100} = e^{-k'lc}.$$

Passing to common logarithms,

$$\log G - \log 100 = -k' l c \log e.$$

Since k', l, and log e are constants, they can be combined to form a new constant K.

Then $\log G - \log 100 = -Kc$

or $\log 100 - \log G = Kc$

or $c = \frac{2 - \log G}{K}$.

L, the density defined as (2-log G), is tabulated against G for different readings of G, and c is calculated from the formula,

$$\frac{L}{K} = c.$$

K is determined for any colored substance which follows Beer's law by calculating values of L for a series of observed G values and plotting the values of L against the corresponding known values of c.

The calculations in Table 1 show the percentage of error due to inaccuracy in reading of one quarter scale division at different points on the scale when aliquot samples are varied so that the reading corresponds in every case to a constant concentration of chromogen in the original material.

Table 1

Percentage error involved in inaccuracy of reading galvanometer

G (galganometer reading)	L_G = 2-log G	$G+\frac{1}{4}$	$L_{G+\frac{1}{4}}$	$\Delta L = L_G - L_{G+\frac{1}{4}}$	% Error = $\frac{\Delta L}{L_G} \times 100$
1.00	2.00000	1.25	1.91309	.08691	4.346
10.00	1.00000	10.25	.98928	.01072	1.072
12.00	.92082	12.25	.91186	.00896	.985
20.00	.69897	20.25	.69357	.00540	.744
25.00	.60206	25.25	.59774	.00432	.718
30.00	.52288	30.25	.51927	.00361	.691
35.00	.45593	35.25	.45284	.00309	.678
38.00	.42022	38.25	.41737	.00285	.678
40.00	.39794	40.25	.39523	.00271	.682
45.00	.34679	45.25	.34438	.00241	.694
50.00	.30103	50.25	.29886	.00217	.722
60.00	.22185	60.25	.22004	.00181	.816
70.00	.15490	70.25	.15335	.00155	1.001
80.00	.09691	80.25	.09555	.00136	1.405
90.00	.04576	90.25	.04455	.00121	2.644
99.00	.00436	99.25	.00327	.00109	24.96

It can be seen that the error is at a minimum of about .7% when the galvanometer reads between 25 and 50, and that the error is less than 1% when the galvanometer reads between 12 and 60.

If G is the correct galvanometer reading for a concentration c of chromogen in the sample, and $L_G = 2 - \log G$, then $L_G/c = K$ for that chromogen. If an error of one quarter scale division is made in reading the galvanometer, then $G+\frac{1}{4}$ is the erratic galvanometer reading, $L_{G+\frac{1}{4}}$ is the corresponding density, and $\left(\frac{L_{G+\frac{1}{4}}}{K}\right) = c'$ the erratic value for the concentration of chromogen obtained. The percentage of error caused by an erroneous galvanometer reading amounting to one quarter scale division is

$$\% \text{ error} = \frac{c - c'}{c} \times 100$$

$$= \frac{\left\{\left(L_G/K\right) - \left(L_{G+\frac{1}{4}}/K\right)\right\}}{L_G/K} \times 100$$

$$= \frac{\left\{\left(L_G - L_{G+\frac{1}{4}}\right)/K\right\}}{L_G/K} \times 100$$

$$= \frac{\left(L_G - L_{G+\frac{1}{4}}\right) \times K \times 100}{K \times L_G}$$

$$= \frac{\left(L_G - L_{G+\frac{1}{4}}\right)}{L_G} \times 100 .$$

These calculations show that the errors of the colorimeter are independent of the value of K; that is, regardless of the size of the proportionality constant between the logarithm of the galvanometer reading and the absolute value of the concentration of chromogen in the sample, the accuracy of the reading can be held constant by diluting or concentrating the

sample, provided that the color follows Beer's law. Thus, if the color developed by a certain reagent from the sodium in 1.0 cc. of blood gives a galvanometer reading of 1, the percentage of error would be 4.35. By using 0.5 cc. of blood, or by using twice the volume to contain the color from 1.0 cc., the reading obtained would be 10, and the percentage of error only 1.07.

It also follows that from the point of view of the colorimetry, there is no choice between two colors which follow Beer's law. Practically, of course, the choice must be limited to a color which gives accurate readings (near the middle of the galvanometer scale) with a conveniently sized sample or a conveniently and accurately obtainable aliquot portion of it.

In order to obtain results of the accuracy indicated in table 1, it is necessary that the colorimetric tubes be rigorously standardized. The tubes used in these experiments were standardized with filter 440, first by selecting tubes which gave identical readings when filled with distilled water, then by further selecting those tubes which gave identical readings when filled with a standard solution of the colored substance to be used in further experiments.

The methods so far proposed for the determination of sodium may be classified into six groups as follows:

1. Methods requiring multiple determinations, such as the methods of McCrudden and Sargent (61), Stoddard (98),

Brown and Shohl (10), and Prinsen-Geerlings (75).

2. The dihydroxytartaric acid method of Fenton (28).

3. Spectrographic methods, as those of Thomson and Lee (91) and Jansen, Heyes, and Richter (38).

4. Methods using bismuth caesium nitrite (5,22).

5. Pyroantimonate methods, as those of Kramer (47), Kramer and Gittleman (48), and Lewin (53).

6. Uranium methods, as those of Blanchetière (9), Barber and Kolthoff (6), and Woelfel (97).

For routine clinical use multiple gravimetric analyses are too tedious. Fenton's (28) method was gravimetric. Since the spectrograph is not a commonly used instrument in clinical laboratories, a spectrographic method would have limited application. Doisy and Bell (22) in their development of the bismuth caesium nitrite method were unsuccessful in their efforts to stabilize the reagents. As a volumetric procedure, Rourke's (79) modification of the Kramer and Gittleman (48) pyroantimonate method seems above reproach. But the instability of arsenious sulfide sols (63) raises a serious objection to the colorimetric pyroantimonate procedure of Yoshimatsu (98). The search for a colorimetric procedure for the determination of sodium is almost necessarily limited to the uranium triple salts.

With one exception, the colorimetric methods which have been developed for the determination of sodium by triple

acetate precipitation have depended on the measurement of the color of some compound of uranium. Woelfel (97) precipitated sodium as the uranyl manganese acetate, oxidized the manganese to permanganate, and measured its color. The presence of large quantities of organic matter in biological fluids and the ease of reduction of permanganate would seem to constitute an objection to the application of Woelfel's method to biological fluids, unless great precautions were taken to destroy all organic matter.

There is no reason for believing that a color reaction which works with the uranium in one of the triple acetate salts would not work equally well with that in any of the others. The choice of precipitant, therefore, was made on the basis of other properties of the individual triple salts. Caley (13) found that the cobalt salt is not suitable as a quantitative reagent for sodium because it does not give complete precipitation. Its suitability as a qualitative reagent depends on its lack of sensitivity to potassium. No results have been published on the use of nickel uranyl acetate as a quantitative reagent for sodium, and it was not studied here. The use of uranyl manganese acetate as the precipitant in a colorimetric method in which the color of a uranyl compound was measured would entail the complete supression of color of the highly colored manganese compounds. Large errors have been found in the

gravimetric determination of sodium as the magnesium uranyl acetate by Kling and Lassieur (43) and by Barber and Kolthoff (6). Even Caley and Foulk (17), who have been the sole users of the magnesium triple salt in this country, admitted that the corresponding zinc salt gives better results with amounts of sodium less than 8 milligrams.

An examination of the literature on uranyl zinc acetate as a sodium reagent showed many advantages in its favor. In concentrated solution it quantitatively precipitates sodium as $(UO_2)_3\ ZnNa(CH_3COO)_9.6H_2O$ provided either that the reagent is first saturated with the sodium salt (6) or that the solubility of the sodium salt in the reagent is decreased by adding alcohol to the reagent(8). The sodium compound, the composition of which does not vary, can be separated from the excess of reagent easily (6). It is freely soluble in water. On the basis of these considerations uranyl zinc acetate was chosen as the precipitant.

Of the various color reactions of the uranyl ion outlined above, those with alizarin and cochineal were given little attention. The use of such colors would involve two major difficulties; first, the standardization of the cochineal or alizarin solutions, and second, the rigid control of acidity to prevent shifting of color characteristics of the excess of dye present in solution. Such difficulties represent unnecessary complications in a routine procedure.

The color of the triple salt itself has been studied by Hoffman and Osgood (36), who found that it is very unstable to heat and acidity. As they pointed out, the color can be stabilized by dissolving the triple salt in tenth-normal ammonium thiocyanate instead of in water. But ammonium thiocyanate is unstable in tenth-normal solution, and the use of this method entails the frequent standardization of the solvent.

The red color of uranyl ferrocyanide has been measured in the visual colorimeter in the methods of Barrenscheen and Messiner (8), Poulsson (74), McCance and Shipp (60), and Salit (82). As a preliminary step in the adaptation of this method to the photoelectric colorimeter, the absorption of light by the red compound was determined spectrophotometrically*. As shown by chart 1, the maximum absorption occurs somewhere in the ultraviolet spectrum. Measurement of light absorption by this substance in the Evelyn colorimeter is open to two objections: first, the colorimeter is not equipped to measure ultraviolet absorption, and by using this instrument one would measure the absorption of light which is not absorbed specifically by the compound; second, the blank is colored and also shows maximum absorption in the visible spectrum at 420 millimicra. Consequently, the ferrocyanide method was discarded.

*Quantitative measurements of light absorption (Charts 1,2, and 3) were made on the recording spectrophotometer at the Color Measurement Laboratory of the Massachusetts Institute of Technology.

Chart 1. Light absorbed by compound of triple salt with potassium ferrocyanide

Preliminary studies of a series of phenolic and sulfhydryl compounds as colorimetric reagents for uranium showed that thioglycollic acid gives an intense red color with uranium. Unfortunately, it has a lingering, mephitic odor which discouraged further use. Tannic and gallic acids showed a strong tendency to form precipitates. Snell and Snell (86) have already mentioned this difficulty. Sodium salicylate and sulfosalicylic acid showed many advantages as colorimetric reagents. Both are very soluble in water, and both give colorless solutions when no uranium is present. Sulfosalicylic acid is a common reagent in clinical laboratories, and sodium salicylate in inexpensive enough to become one. The intensity of color formed by either reagent from the triple salt precipitated from .1 or .2 cc. of serum is such that the solution can be diluted to 100cc. to obtain galvanometer readings which fall in the most accurate part of the scale. Both colors follow Beer's law sufficiently well to allow application of the law over limited ranges of concentration. Neither color changed appreciably in intensity during three hours. The color with sodium salicylate showed a marked sensitivity to acetic acid and to sodium acetate. Sodium acetate is necessary for the development of color by sulfosalicylic acid. Spectrophotometric examination of the two colors (see Charts 2 and 3) showed that each has a narrow absorption band in the visible spectrum. With sodium

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tories, and sodium salicylate is inexpensive enough to
become one. The intensity of color formed by either reagent
from the trivalent salt precipitated from .1 or .2 cc. of serum
is such that the solution can be diluted to 100cc. to obtain
galvanometer readings which fall in the most accurate part
of the scale. Both colors follow Beer's law sufficiently
well to allow application of the law over limited ranges of
concentration. Neither color changed appreciably in inten-
sity during three hours. The color with sodium salicylate
showed a marked sensitivity to acetic acid and to sodium
acetate. Sodium acetate is necessary for the development of
color by sulfosalicylic acid. Spectrophotometric examination
of the two colors (see Charts 2 and 3). showed that each has
a narrow absorption band in the visible spectrum. With sodium

Chart 2. Light absorbed by compound of triple salt with sodium salicylate

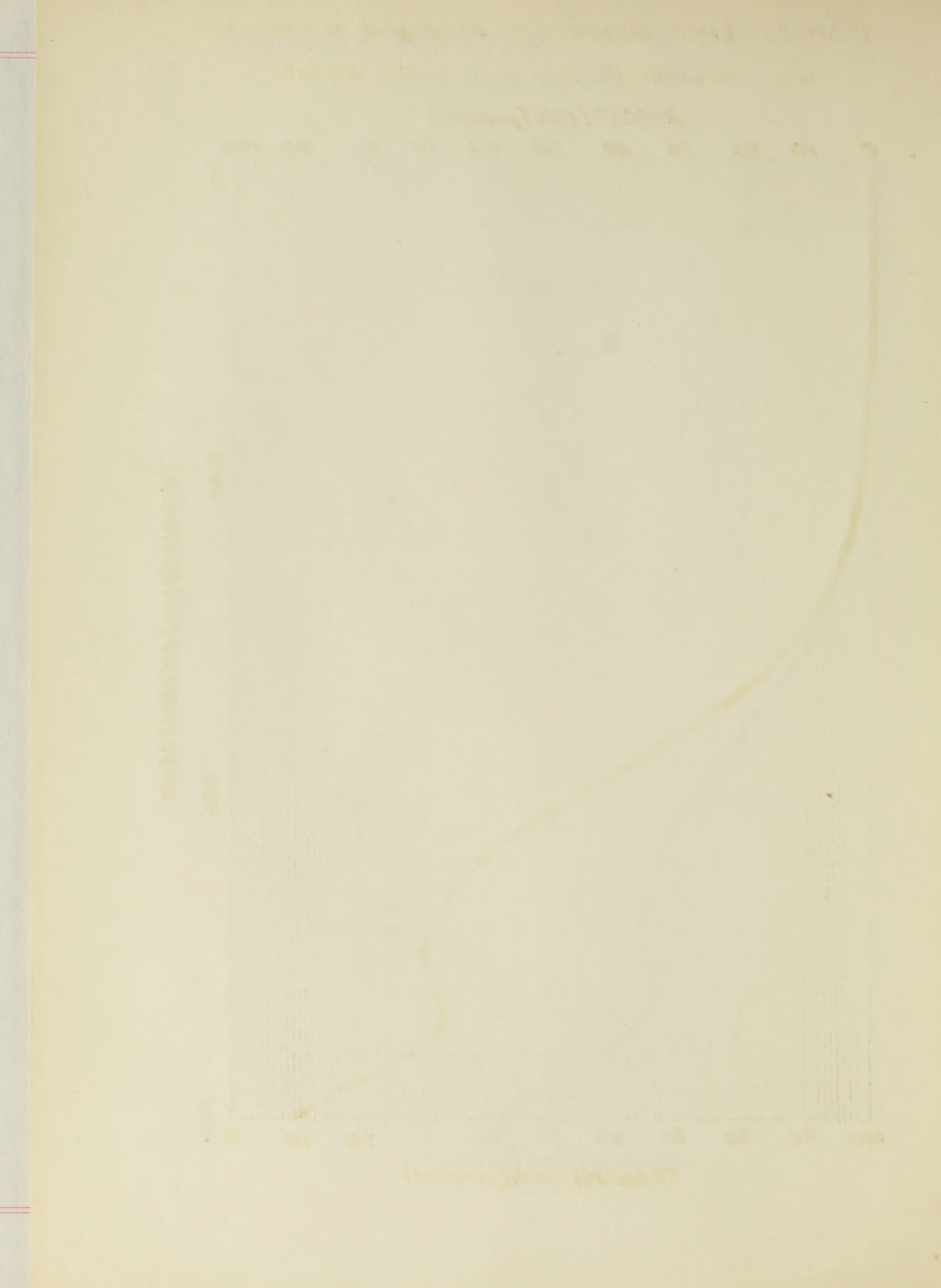

Chart 3. Light absorbed by compound of triple salt with sulfosalicylic acid and sodium acetate

salicylate the maximum occurs at 465 millimicrons, with sulfosalicylic acid and sodium acetate, at 455 millimicrons. Since the salicylate color is so sensitive to acetates; since the absorption band of the sulfosalicylic acid color is more pronounced; and since it coincides more closely with a standard filter of the Evelyn colorimeter (maximum transmission, 440 millimicrons; range of transmission, 410-475 millimicrons), sulfosalicylic acid was chosen as the reagent for color development.

Development of method

In order to be able to estimate the errors in any particular step of the final method, the calibration curves for the color and the experiments in the development of the method were carried out on the basis of a standard solution prepared from uranyl acetate, zinc acetate, and sodium acetate, rather than on the basis of the triple salt precipitated from a standard solution of sodium chloride. The primary standard was prepared by dissolving in 500 cc. of water; 9.1308 gm. of uranyl acetare (Merck's "Reagent" $UO_2(CH_3COO)_2 \cdot 2H_2O$, lot 33008, assay 100.0%; 1.5745 gm. of zinc acetate (Merck's "Reagent" $Zn(CH_3COO)_2 \cdot 2H_2O$; and 0.9763 gm. of sodium acetate (Merck's "Reagent" $NaCH_3COO \cdot 3H_2O$). This is equivalent to a solution containing 22.0706 gm. of $(UO_2)_3ZnNa(CH_3COO)_9 \cdot 6H_2O$ per liter; 1.0 cc. contains 0.330 gms. of sodium.

This artificial standard solution was chosen in preference to a standard solution of a weighed quantity of

crystallized uranyl zinc sodium acetate for the following reasons. 1. The preparation of the triple salt by the method of Hoffman and Osgood (36) is wasteful of reagent, requiring a waste of uranium of over three times the weight of that recovered in the triple salt. 2. The method of McCance and Shipp (60) is less wasteful, yielding about 42% recovery, but a pure product could not be prepared by this method due to the reduction of uranium by the large amount of alcohol present.* A yellow precipitate was obtained, giving a color with sulfosalicylic acid 2.2% less intense than that of an equal concentration of the synthetic standard described above.

In the standardization of the color obtained from the triple salt with sulfosalicylic acid, two standard solutions were prepared and were diluted 1:20 with water. Varying amounts of these solutions were added to 100 cc. volumetric flasks and diluted to about 70 cc. with distilled water. Then were added in order ** 4 cc. of 5% sulfosalicylic acid, 4 cc. of 10% sodium acetate, and water to make 100 cc. The flasks were shaken and about 15 cc. of the contents transferred to Evelyn colorimeter tubes previously rinsed with the solution. The tubes were read in the colorimeter with filter 440 mμ and the values of K calculated from the known concentrations of sodium present and the observed galvanometer readings. The

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* Lattimer and Hildebrand (52) mention this ease of reduction of uranyl salts by alcohol.
** Snell and Snell (86) state that a more intense color is obtained by adding the phenolic acid before the sodium acetate.

first four columns of tables 2 and 3 indicate the results of these experiments, and chart 4 shows the values of L plotted against the concentration of sodium in the sample. These tables indicate that the variations in the galvanometer readings for any particular concentration of sodium did not vary by more than one quarter of a scale division. By calculating $\Delta L/L$ as in table 1, it can be shown that the only instance in which this difference amounted to more than one percent was at the concentration of .132 mgms per 100cc., where the percentage difference is $\frac{.1163 - .1149}{.1163} \times 100$, or 1.2%.

Column 5 of tables 2 and 3 and chart 4 show that Beer's law does not apply exactly throughout the range of concentrations investigated. Instead of constant values of K in the tables and a straight-line-plot of L against C in chart 4, the values of K show a constant downward trend with increasing concentration of color. The cause of this decrement of color intensity with increasing concentration was not investigated.

The decrease of K with increasing values of L was studied for the two standard solutions in the manner indicated by tables 4 and 5 and charts 5 and 6. Values of K, as calculated from the equation

$$K = L/C$$

were plotted against the corresponding values of L. The

Chart 4. Conformity with Beer's law of color with sulfosalicylic acid and sodium acetate

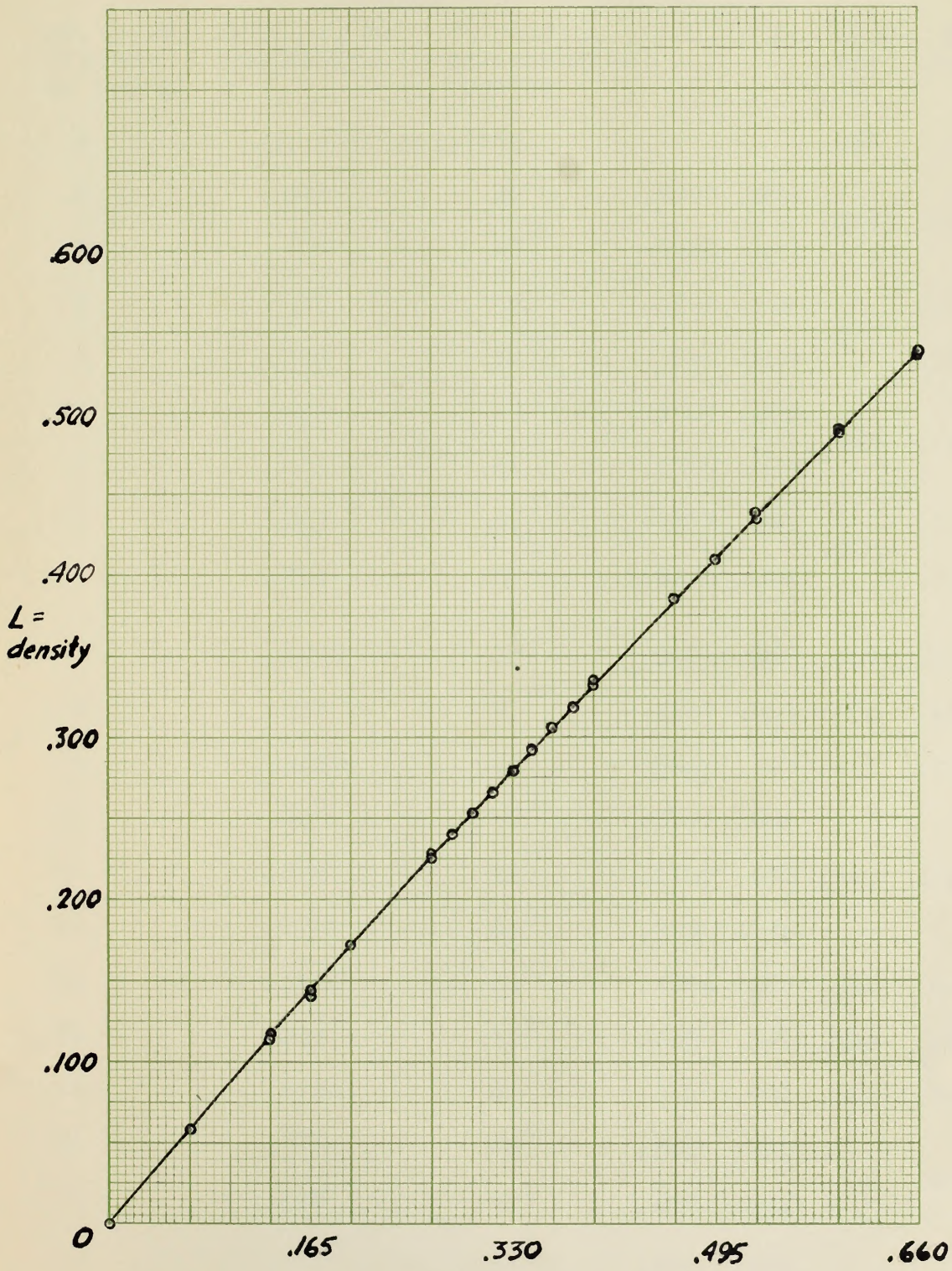


Table 2

Calibration of galvanometer

cc. soln. diluted to 100cc.	Conc. of Na (mgm/100cc.)	Corrected galvanometer reading	L	K	Average K	K calc.	% difference
0	By extrapolation				.889		
4	.066	87^{2}	.0580	.879			-.2
4	.066	87^{2}	.0580	.879	.879	.881	-.2
8	.132	76^{2}	.1163	.881		.872	+1.0
8	.132	76^{3}	.1149	.870	.876	.872	-.2
12	.198	67^{2}	.1707	.862			-.2
12	.198	67^{2}	.1707	.862	.862	.864	-.2
16	.264	59^{1}	.2273	.861		.856	+.6
16	.264	59^{2}	.2255	.854	.858	.856	-.2
17	.2805	57^{2}	.2403	.857	.857	.854	+.4
18	.297	55^{3}	.2537	.854	.854	.852	+.2
19	.3135	54^{1}	.2656	.847	.847	.850	-.4
20	.330	52^{2}	.2798	.848			-.1
20	.330	52^{2}	.2798	.848	.848	.849	-.1
20	.330	52^{2}	.2798	.848			-.1
21	.3465	51^{0}	.2924	.844	.844	.847	-.4
22	.363	49^{2}	.305	.840	.840	.845	-.6
23	.3795	48^{0}	.319	.841	.841	.843	-.2
24	.396	46^{2}	.332	.838			-.4
24	.396	46^{2}	.332	.838	.838	.841	-.4
28	.462	41^{1}	.385	.833			-.1
28	.462	41^{1}	.385	.833	.833	.834	-.1

Table 2

Table 2 (cont.)

Calibration of galvanometer

cc. soln. diluted to 100cc.	Conc. of Na (mgm/100cc)	Corrected galvanometer reading	L	K	Average K	K calc.	o/o difference
32	.528	36^{2}	.438	.830	.827	.826	+.5
32	.528	36^{3}	.435	.824		.826	-.2
36	.594	32^{2}	.488	.822	.819	.818	+.5
36	.594	32^{3}	.485	.816		.819	-.4
40	.660	29^{0}	.538	.815	.812	.811	+.5
40	.660	29^{1}	.534	.809		.812	-.4

Table 3

cc. soln. diluted to 100cc.	Na mgm/100cc.	Reproducibility of color G	L	K	K value of independently prepared solution Obs.	Calc.	% difference of K values from Obs.	Calc.
0	By extrapolation			.895	(ave.)	.889		
8	.132	76^2	.116	3.881		.872	+.6	+1.0
		76^2	.116	3.881		.872	+.6	+1.0
		ave.		.881	.876			
10	.165	71^3	.144	2.874		.868		+.7
		71^3	.144	2.874		.868		+.7
		72^0	.142	7.865		.868		-.3
		ave.		.871				
16	.264	59^2	.225	5.854		.856	-.5	-.2
		59^1	.227	3.861		.856	+.4	+.6
		ave.		.858	.858			
20	.330	52^2	.279	8.848		.849	.0	-.1
		52^2	.279	8.848		.849	.0	-.1
		52^2	.279	8.848		.849	.0	-.1
		ave.		.848	.848			
24	.396	46^1	.335	.846		.840	+.9	+.7
		46^2	.332	.838	.838	.841	.0	-.4
		ave.		.842				
30	.495	39^0	.409	.826		.830		-.5
		39^0	.409	.826		.830		-.5
		39^0	.409	.826		.830		-.5
		ave.		.826				

Table 3 (cont.)

Reproducibility of color

cc. soln. diluted to 100cc.	Na mgms/100cc.		G	L	K	K value of independently prepared solution		% difference of K values from	
						Obs.	Calc.	Obs.	Calc.
32	.528	36^2	.438	.830			.826	+.4	+.5
		36^3	.435	.824			.826	-.4	-.2
			ave.	.827	.827				
40	.660	29^0	.538	.815			.811	+.4	+.5
		29^1	.534	.809			.812	-.4	-.4
			ave.	.812	.812				

Chart 5. Variation of $\frac{\text{density}}{\text{concentration}}$ with density.

First solution

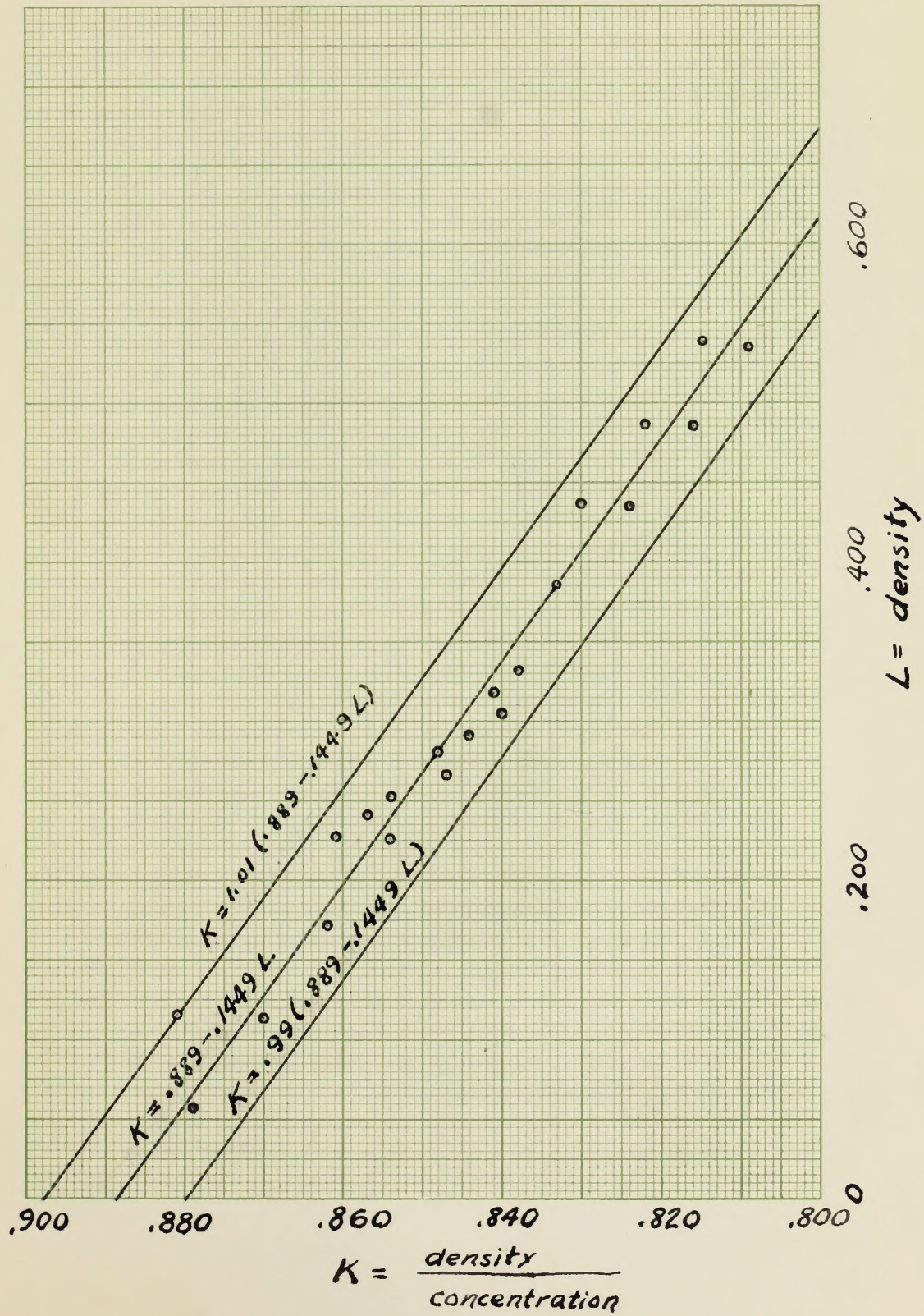


Table 4

Variation of K with L. First solution

Frequency	L	K	LK	L^2
2	.0580	.879	.05098	.00336
2	.1156	.876	.10127	.01336
2	.1707	.862	.14714	.02914
2	.2264	.858	.19425	.05126
1	.2403	.857	.20594	.05774
1	.2537	.854	.21666	.06436
1	.2656	.847	.22496	.07054
3	.2798	.848	.23727	.07829
1	.2924	.844	.24679	.08550
1	.305	.840	.25620	.09303
1	.319	.841	.26828	.10176
2	.332	.838	.27822	.11022
2	.385	.833	.32071	.14823
2	.437	.827	.36140	.19097
2	.487	.819	.39885	.23717
2	.536	.812	.43523	.28730

Σ N = 27 ΣL = 8.0108 ΣK = 22.835 ΣLK = 6.70673 ΣL^2 = 2.84982

Calculations

$27a + 8.0108b = 22.385$
$8.0108a + 2.8498b = 6.7067$
$27/8.0108 = 3.3704$

$27a + 8.0108b = 22.835$
$27a + 9.6050b = 22.604$

$-1.5942b = .231$
$b = -.1449$

$27a - 8.0108 \times .1449 = 22.835$
$27a - 1.161 = 22.835$
$27a = 23.996$
$a = .889$

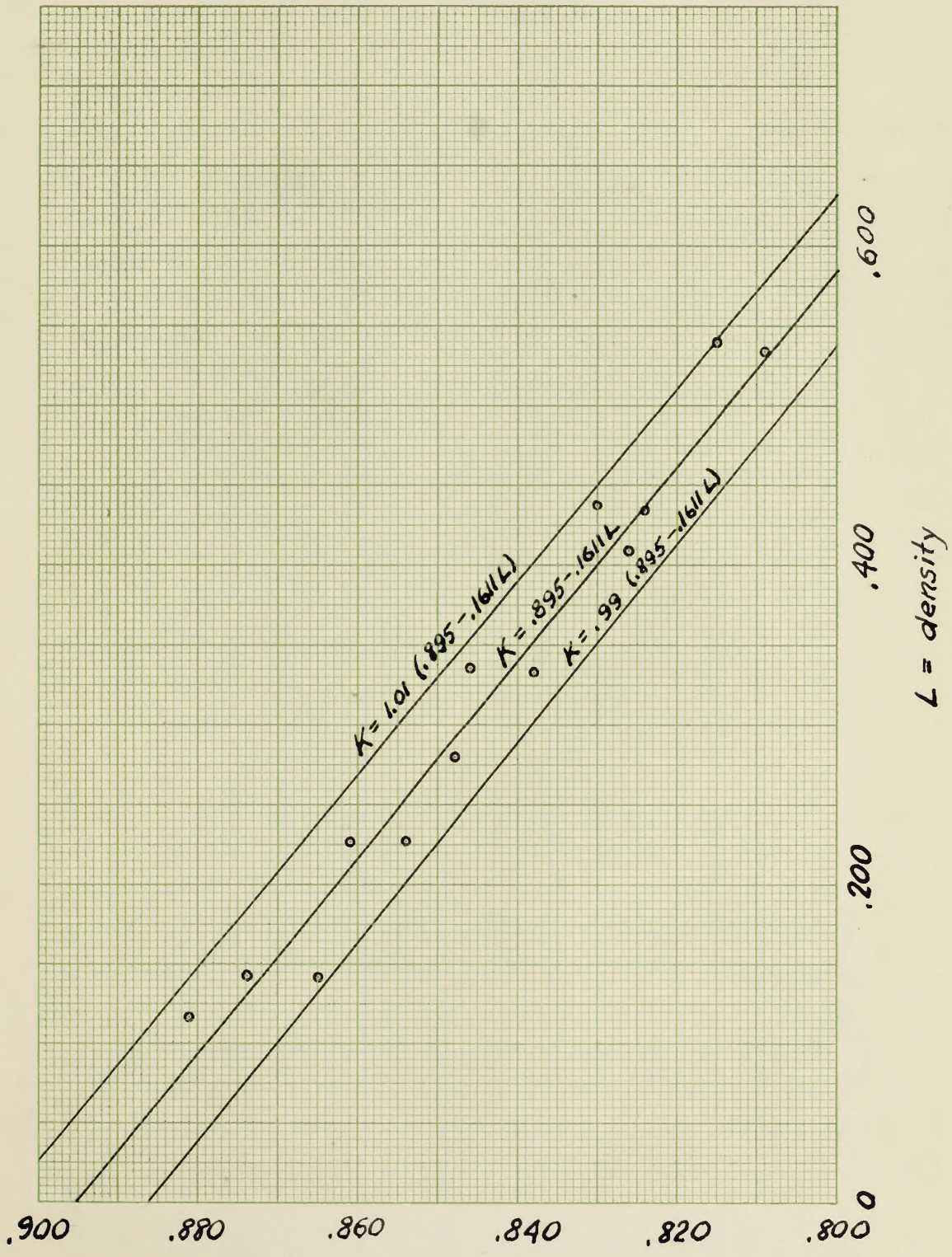
Check
$8.0108 \times .889 - 2.8498 \times .1449 = 6.7067$
$7.1216 - .4129 = 6.7067$
$6.7087 = 6.7067$

Equation: $K = .889 - .1449L$

Line: when L = 0, K = .889
when L = .500,
K = .889 - .500 x .1449
= .889 - .07245
= .817

Chart 6. Variation of density with density.
concentration

Second solution



$$K = \frac{\text{density}}{\text{concentration}}$$

Table 5

Variation of K with L. Second solution

Frequency	L	K	LK	L^2
2	.1163	.881	.10246	.01353
2	.1442	.874	.12603	.02079
1	.1427	.865	.12344	.02036
1	.2255	.854	.19258	.05085
1	.2273	.861	.19571	.05167
3	.2798	.848	.23727	.07829
1	.335	.846	.28341	.11223
1	.332	.838	.27822	.11022
3	.409	.826	.33783	.16728
1	.438	.830	.36354	.19184
1	.435	.824	.35844	.18923
1	.538	.815	.43847	.28944
1	.534	.809	.43201	.28516

Σ n =19 ΣL =5.7949 ΣK =16.074 ΣLK=4.8481
ΣL^2 =2.10635

Calculations

$19a + 5.7949b = 16.074$
$5.7949a + 2.10635b = 4.8481$
$19/5.7949 = 3.2787$

$19a + 5.7949b = 16.074$
$19a + 6.9061b = 15.895$
$-1.1112b = .179$
$b = -.1611$

$19a - 5.7949 \times .1611 = 16.074$
$19a - .934 = 16.074$
$19a = 17.008$
$a = .895$

Check:
$5.9749 \times .895 - 2.10635 \times .1611 = 4.8481$
$5.1864 - .3393 = 4.8481$
$4.8471 = 4.8481$

EQUATION : $K = .895 - .1611 L$

LINE: when L =0, K = .895
when L = .500,
K = .895 - .500 x .1611
= .895 - .081
= .814

equations for the values of K as functions of L were calculated for the two solutions by the method of least squares (70), and the lines corresponding to the derived equations were drawn in. As shown by the lines drawn in at values of K one percent greater or less than those calculated, these equations represent the observed data with a maximum inaccuracy of one percent. Columns 5,6,7, and 8 of table 2 show the actual percental difference between the observed and calculated values of K for one of the solutions.

Since the color does not follow Beer's law exactly, three methods are available for the calculation of the concentration of sodium in an unknown solution; any one of the three can be used to give results of the necessary accuracy.

(1) From the value of L derived from the galvanometer reading, the corresponding value of K can be read from chart 5, or can be calculated from the equation for the relationship of K to L. Then by dividing L by K, the figure for the concentration C is obtained.

(2) The value of C can be read directly from chart 4, the empirical curve drawn throuth the plot of observed values of L against the corresponding values of C.

(3) This method is based on the observed fact (column 5, tables 2 and 3), that over a range of ± 10% in the value of L (and consequently the value of C), the value of K changes by only 1%. This statement holds true particularly well for concentrations of sodium where the corresponding

galvanometer readings fall near the middle of the scale. A deviation of ± 4% in the sodium content of blood serum is clinically significant (36). A 2% error in the determination when the sodium content is 10% low would not be significant. The point in question is always whether or not the sodium level is significantly altered in comparison with the normal: if it is, the exact degree of alteration is relatively unimportant. In dealing with the sodium in blood, one may assume that Beer's law holds, and that

$$C = \frac{L}{K_n},$$

where C is the concentration of sodium in the blood, L is the density corresponding to the observed galvanometer reading, and K_n is the experimentally determined value of the ratio of L to C of a solution whose sodium content is equal to the mean normal value of blood sodium. Chart 7 shows the validity of these assumptions as applied in range of concentrations of .297 to .363 mgms.% of sodium, and that the errors within this range did not exceed 1%.

The dotted line on chart 7 was drawn on the assumption that within the limited range of concentrations indicated, the increase of concentration is directly proportional to the increase in density. This assumption was borne out experimentally, as shown by the close proximity of the experimental points to the line. The calculation* of concentrations from observed densities by this relationship is too ponderous for everyday

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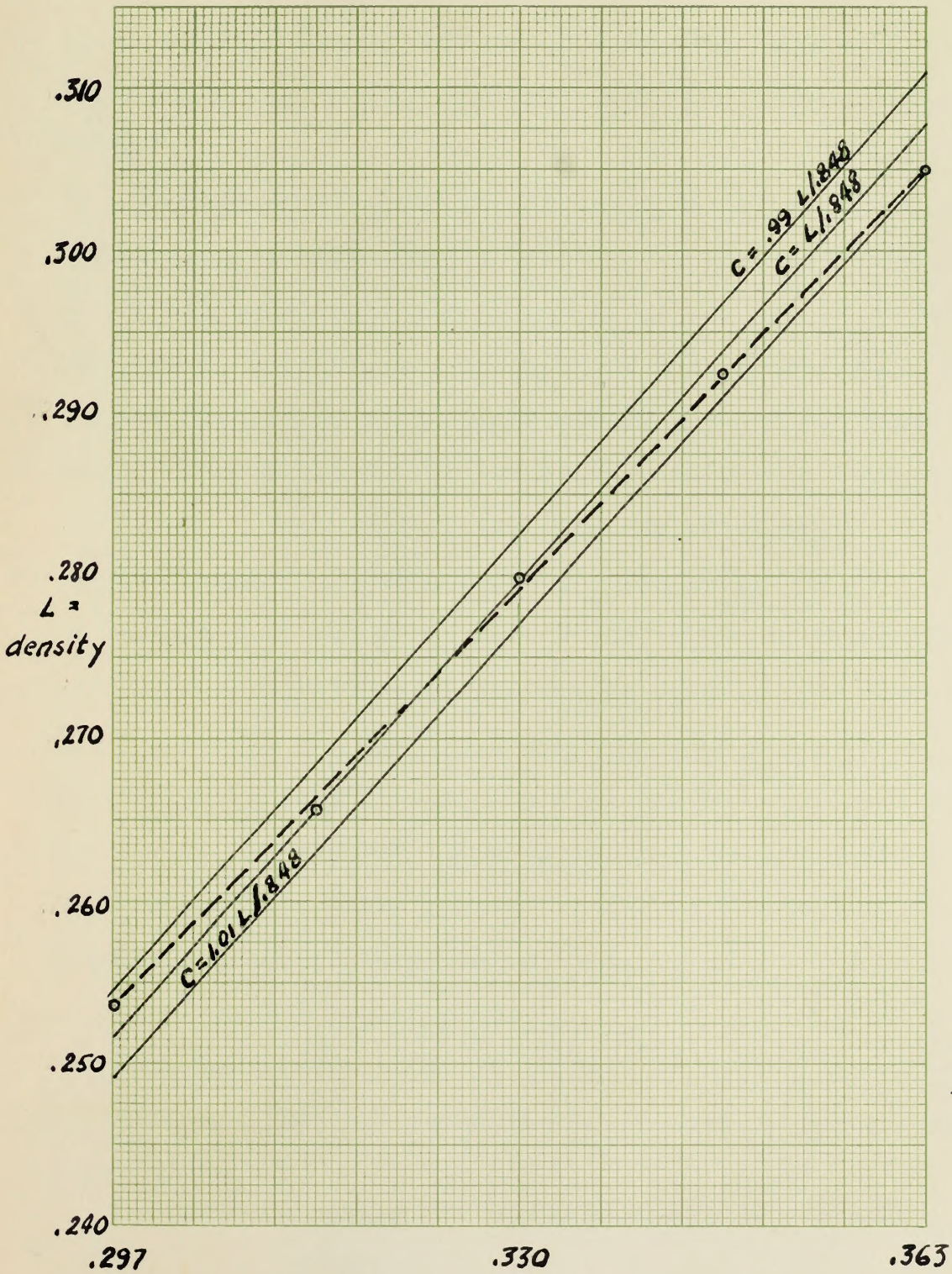
* Where C_H and C_L are the concentrations and L_H and L_L are the

Chart 7. Conformity with Beer's law over short range of concentration



C = milligrams of sodium in 100 cc. of colored solution

work. The relationship is of value in connection with method 2, as outlined above, because it shows that the line on chart 4 can be drawn in small straight segments without serious error.

The reproducibility of the color is indicated by the duplication of galvanometer readings of equivalent dilutions of the two solutions, shown in tables 2 and 3. As a check on the reproducibility, the relation of K to L was studied for the two solutions, as shown in tables 4 and 5 and charts 5 and 6. The equations for the relationship were found to be

$$K = .889 - .1449L \text{ for one solution}$$

and $K = .895 - .1611L$ for the other.

In columns 5,6, and 8 of table 3 the values of K observed for varying concentrations of the second solution are compared

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corresponding densities of the high and low extremes, respectively, over which the principle of the proportionality of increments is to be applied, and L is the density whose concentration C is to be determined,

$$C = C_L + \frac{(L - L_L)}{(L_H - L_L) / (C_H - C_L)} .$$

For example, from the experimental figures in table 2 and chart 7,

$$C_{.2924} = .297 + \frac{(.2927 - .2537)}{(.305 - .2537) / (.363 - .297)}$$

$$C_{calc.} = .347.$$

Concentration taken = .3465 .

with the average of observed K values for corresponding concentrations of the first solution. The limits of variation were found to be from -.5% to +.9%, the average variation ± .33%. In columns 5,7, and 9 of table 3, the values of K observed for dilutions of the second solution are compared with the values expected from the observed values of L and the K-L regression equation of the first solution. The limits of variation were found to be from -.5% to 1.0%, the average variation ± .48%.

In the study of the stability of the color with respect to time, readings were made at intervals on tubes containing about 15 cc. of varying dilutions of the triple salt, made up in every case with 4 cc. of 5% sulfosalicylic acid and 4 cc. of 10% sodium acetate per 100cc. The liquids were left in the tubes between readings, and the standardized tubes were not used throughout; consequently, the readings for any particular concentration do not necessarily check either with one another or with the readings in tables 2 and 3. Results are shown in table 6.

No changes in color were found within the first three hours, but decreases of galvanometer readings implying increases of color corresponding to about 1% were observed in most tubes after ten hours.

Table 7 shows the results of experiments testing the effect of temperature on the color of the solution. Solutions containing the indicated concentration of triple salt

Table 6

Permanence of the color

Na conc. (mgms. %)	Corrected Galvanometer readings immediately	after 1 hour	after 3hours	after 10 hours
.033	93^{1}	93^{1}		93^{1}
.066	87^{1}	87^{1}		87^{1}
.165	71^{3}	71^{3}		71^{3}
	71^{3}		71^{3}	
	71^{3}		71^{3}	
	72^{0}		72^{0}	
	71^{3}			71^{3}
	71^{3}			71^{3}
	72^{0}			72^{0}
.330	52^{2}	52^{2}		52^{1}
	52^{2}		52^{2}	
	52^{2}		52^{2}	
	52^{2}		52^{2}	
	52^{2}			52^{1}
	52^{2}			52^{1}
	52^{2}			52^{1}
.495	39^{1}	39^{1}		39^{0}
	39^{0}		39^{0}	
	39^{0}		39^{0}	
	39^{0}		39^{0}	
	39^{0}			38^{3}

Table 6

Permanence of the color

Na conc. (mgms. %)	Corrected Galvanometer readings		
	Immediately	after 1 hour	after 3hours	after 10 hours
.033	93_1	93_1		93_1
.066	87_1	87_1		87_1
.165	71_2	71_2	71_2	71_2
	71_2			
	71_2			
	72_0		72_0	
	71_2			
	71_2			71_2
	72_0			72_0
.330	82_2	82_2		82_1
	82_2		82_2	
	82_2		82_2	
	82_2		82_2	
	82_2			
	82_2			82_1
	82_2			82_1
.495	29_1	29_1		29_0
	29_0		29_0	
	29_0		29_0	
	29_0		29_0	
	29_0			
	26_3			

Table 6 (Cont.)

Permanence of the color

Na conc. (mgms.%)	Corrected Galvanometer readings immediately	after 1 hour	after 3 hours	after 10 hours
.495	39^{0}			38^{3}
	38^{3}			38^{2}
.660	29^{3}	29^{3}		29^{2}

Table 7

Effect of temperature on the color

Na mgms.%	Corrected galvanometer readings at 17.5°C.	20.5°C.	25.3°C.	30.5°C.
.165	72^{0}	71^{3}	71^{3}	71^{3}
	72^{1}	72^{1}	72^{0}	72^{0}
	72^{0}	72^{0}	71^{3}	71^{3}
.330	52^{3}	52^{3}	52^{2}	52^{2}
	53^{0}	53^{0}	52^{2}	52^{2}
	52^{3}	52^{3}	52^{1}	52^{2}
.495	39^{1}	39^{1}	39^{0}	38^{3}
	39^{1}	39^{1}	39^{0}	39^{0}
	39^{1}	39^{1}	39^{0}	39^{0}

Table 6 (Cont.)

Permanence of the color

No conc. (mgms.%)	Corrected Galvanometer readings immediately	after 1 hour	after 5 hours	after 10 hours
.495	39_0			38_3
	38_3			36_5
.660	29_3	29_3		29_5

Table 7

Effect of temperature on the color

No mgms.%	Corrected galvanometer readings at 17.5°C.	20.6°C.	26.3°C.	30.5°C.
.165	72_0	71_8	71_8	71_5
	72_1	72_1	72_0	72_0
	72_0	72_0	71_8	71_5
.330	52_3	52_3	52_2	52_2
	52_0	52_0	52_2	52_2
	52_3	52_3	52_1	52_2
.495	39_1	39_1	39_0	38_3
	39_1	39_1	39_0	39_0
	39_1	39_1	39_0	39_0

plus 4 cc. of each of 5% sulfosalicylic acid and 10% sodium acetate were prepared in 100 cc. volumetric flasks. The flasks were placed in a water bath at the temperature indicated for 20 minutes. About 15 cc. of the contents were transferred to Evelyn tubes rinsed with the solution, and the tubes were immediately replaced in the water bath. The outside of the tubes were dried and the tubes read in the colorimeter. Immediately after reading the temperature of the solution in each tube was taken. The results in table 7 indicate a barely detectable decrease in intensity (.7-1.0%) when the temperature is 20° or below from that observed at 25° and above. As the room temperature varied from 24° to 26.5° during the course of the experiments reported in this paper, this temperature effect was neglected. If the room temperature varied markedly or fell below 23°, it would, of course, be necessary to apply a correction factor of plus one quarter scale division to those readings from 72 to 36, more or less, taken below this temperature.

Hoffman and Osgood (36) reported that the effect of temperature on the color of a pure solution of the triple salt is so marked that the heat of the colorimeter lamp affects it. No such sensitivity was detected in the color developed from the triple salt by sulfosalicylic acid. Three tubes gave identical galvanometer readings before and after being exposed to the heat of the lamp for two minutes-more

than 4 times the normal length of time required for making a reading in the photoelectric colorimeter.

The color developed by sulfosalicylic acid was found to be quite sensitive to changes of acidity. The addition of mineral acid diminished the color markedly. The dropwise addition of sodium hydroxide increased the color to a density about twice as great as that used in these experiments; further additions caused rapid fading of the color. The most intense color could be obtained by neutralizing the sulfosalicylic acid to a phenolphthalein end point before adding it to the uranyl solution. The pH of the colored solutions with sulfosalicylic acid and sodium acetate varied from 4.5 to 5.3 with increasing concentrations of uranium; the pH of solutions with neutralized sulfosalicylic acid varied from 9.1 to 5.5 with increasing concentrations of uranium. The more intense color was not used in further experiments because it was found to be much more sensitive to changes of acidity than was the color with sulfosalicylic acid and sodium acetate, and because it did not follow Beer's law nearly as well as did the original color. The density of the original color increased 94% between concentrations of sodium of .165 mgms. per 100 cc. and .330 mgms. per 100 cc.; over the same range, the density of the color with neutralized sulfosalicylic acid increased only 69%.

An experiment was performed to determine the effect of changing the relative and absolute amounts of sodium acetate and sulfosalicylic acid. To each of twelve 100 cc. volumetric flasks was added 20 cc. of triple salt solution containing .330 mgms. of sodium. Varying amounts of sulfosalicylic acid and sodium acetate were added, and the contents diluted to volume and read in the colorimeter. Results are shown in table 8.

Increasing the amounts of sodium acetate and sulfosalicylic acid had no effect on the color as long as the ratio of the two reagents was not varied. Increasing the ratio of sodium acetate to sulfosalicylic acid caused a decrease of intensity.

On the basis of these results, precautions were taken to measure accurately the color reagents and to make subsequent solutions of sulfosalicylic acid of equal concentration to that of the solution used in standardization (5 cc. of sulfosalicylic acid solution required 17.25 cc. 0.1000 N NaOH to neutralize it to a phenolphthalein end point).

Because of the solubility of uranyl zinc sodium acetate in water, it is necessary to take special precautions to insure completeness of precipitation of the sodium in any specimen. The precipitating reagent is made very concentrated in uranyl zinc acetate, but the volume of the sample solution reduces this somewhat. Two general methods have been proposed for insuring more complete precipitation. In their

Table 8

Effect of varying amounts of reagents on color density.

Na Mgms.%	5% Sulfosalicylic acid added cc.	10% NaAc added cc.	Corrected galvanometer reading	Density
0	4.0	4.0	Central setting 73^3	
.330	4.0	4.0	52^2	.2798
	4.0	4.0	52^2	.2798
	3.7	4.0	52^3	.2777
	3.7	4.0	52^3	.2777
	3.7	4.3	53^1	.2736
	3.7	4.3	53^1	.2736
	3.7	3.7	52^2	.2798
	3.7	3.7	52^2	.2798
	4.0	3.7	51^2	.2882
	4.0	3.7	51^3	.2861
	4.3	4.3	52^2	.2798
	4.3	4.3	52^2	.2798

Table 8

Effect of varying amounts of reagents on color density.

original procedure, Barber and Kolthoff (6) saturated the reagent with the triple salt and filtered immediately before using the reagent. Butler and Tuthill (12), Ball and Sadusk (4), and Hoffman and Osgood (36) used this method. Barrenscheen and Messiner (8) did not saturate the reagent with the triple salt, but used alcohol in the precipitating mixture. McCance and Shipp (60), Snell and Snell (86), Salit (82), Jendrassik and Dziobek (39), Rusznyák and Hatz (81), Weinbach (96), Chen (19), and Marenzi and Gerschmann (55,56) used alcohol in amounts varying from 11% to 50% of the total volume of sample solution plus precipitants.

Holmes and Kirk (37), using the saturated precipitant, found it to be necessary to carry out the precipitation in a refrigerator to insure constancy of values, although Ball and Sadusk (4) worked at room temperature. Hoffman and Osgood (36) pointed out that precipitation is not absolutely complete with the saturated reagent because of the dilution of the reagent by the sample solution.

Regarding the use of alcohol, it has already been pointed out (p. 26) that uranyl salts are unstable in the presence of alcohol. Thus it is necessary to add the alcohol at the time of precipitation. Snell and Snell (86) reported 33% as the optimal concentration of alcohol in the precipitating mixture, but Marenzi and Gerschmann (56) stated that 25% is the highest concentration which can be used without precipitating some of the uranyl zinc acetate. The procedure of

Weinbach (96), in which 2.1 cc. of 95% alcohol is added in small portions to 1 cc. of sample and 5 cc. of reagent, was used in the experiments summarized in table 9.

The triple acetate precipitate must be washed free from adherent reagent before it can be determined accurately by any of the procedures so far devised. Barber and Kolthoff (6) and Butler and Tuthill (12) used a saturated alcoholic solution of uranyl zinc sodium acetate for washing out the excess of reagent. Hald (35), Salit (82), and Ball and Sadusk (4) found this reagent to be unsatisfactory because of the decomposition of the uranium in the solution. Salit (82) introduced the use of a saturated solution of triple salt in glacial acetic acid for the first washing; he then washed out the acetic acid with ether. Marenzi and Gerschmann (55,56), Chen (19), and Ball and Sadusk (4) found these to be satisfactory wash reagents. Weinbach (96) washed the precipitate once with a saturated solution of the triple salt in acetone. In attempting to use this reagent in the experiments here reported, it was found that the galvanometer readings obtained were much lower than the theoretical values for standard triple salt solutions. This would indicate that the wash reagent precipitates, rather than dissolves, the excess of reagent. By mixing the wash liquid with the precipitant, this surmise was verified.

Hoffman and Osgood (36) found that all three of the wash reagents mentioned produced precipitates of triple salt when

brought in contact with the precipitant, and that the acetic acid reagent produced the least precipitate. They also observed that the pure liquids produced no precipitate. Because of the irritating odor of glacial acetic acid, they used a 15% solution of acetic acid in alcohol, saturated with the triple salt.

By decreasing the solubility of uranyl zinc sodium acetate in acetic acid with the addition of ethyl acetate, it was possible in these experiments to avoid the use of saturated wash liquids and to evade the obvious limitations of such reagents. About 10 mgm. samples of uranyl zinc sodium acetate were shaken with 5 cc. each of ethyl alcohol, 15% acetic acid in alcohol, glacial acetic acid, 10% ethyl acetate in acetic acid, and ether. After allowing the excess of triple salt to settle, a few drops of the supernatant fluids were mixed with an equal volume of potassium ferrocyanide solution. The solubility of the triple salt in the liquids, as estimated by the intensity of red color produced with ferrocyanide, was found to decrease in the order above mentioned.

Acetic acid solutions containing 0, 20, 40, 60, and 80% by volume of ethyl acetate were shaken with equal volumes of uranyl zinc acetate reagent (96) and with 10 mgm. samples of the triple salt. Glacial acetic acid and the 20% ethyl acetate solution gave no precipitate with the reagent. The 40% solution produced a slight precipitate in ½ hour, the 60%

solution immediately. The mixture with the 80% solution separated into a solid and 2 liquid phases. The supernatant fluids from the triple salt were tested with ferrocyanide as before. The solutions containing 40% or more ethyl acetate gave no color.

A solution containing 30% of ethyl acetate in acetic acid was found to give no color with ferrocyanide after shaking with triple salt, and to give no precipitate with an equal volume of uranyl zinc acetate reagent. Although this solution has a very irritating odor, it can be used without discomfort by delivering it from a wash bottle if a third hole, containing a short glass tube, is put through the stopper and a Bunsen valve attached to the inner end of the mouthpiece. The flow of liquid is regulated by placing the index finger over the third tubing.

The efficiency of precipitation and washing was tested by analyzing standard solutions of sodium chloride. Varying amounts of standard solution of sodium chloride were delivered into centrifuge tubes from calibrated intervals of a microburette and diluted to 1 cc.; 5 cc. of uranyl zinc acetate reagent, prepared according to Weinbach (96) were added, followed by seven .3cc portions of 95% ethyl alcohol during ½ hour. The tubes were centrifuged for 10 minutes at 2000 r.p.m., decanted and inverted over a piece of cheesecloth for 5 minutes. The mouths of the tubes were then wiped dry, and into each was blown about 1 cc. of 30% ethyl acetate in

acetic acid. The precipitate was agitated by holding the tube firmly between the thumb and index finger of the left hand and tapping the base with the right forefinger, thus producing a rotatory motion of the liquid in the tube. The walls of the tubes were then washed down with about 2 cc. more of liquid. The tubes were again centrifuged for 10 minutes, decanted, inverted for five minutes, and wiped as before. The precipitate was then washed twice with 5 cc. portions of ether to remove the acetic acid, which interferes with the color with sulfosalicylic acid. After ether washings the tubes were centrifuged for only 5 minutes and drained for only one minute. Longer draining after ether washings may allow the precipitate to become so dry that it falls out of the tube. After draining from the second ether washing, the tubes were placed over a steam radiator for 5 minutes to dry. The contents of each tube were dissolved by agitating with 3-4 cc. of water, transferred quantitatively to a 100 cc. volumetric flask, and diluted to about 70 cc. Then were added 4 cc. of 5% sulfosalicylic acid solution and 4 cc. of 10% acetic acid solution, and the solutions diluted to volume and mixed. A 15 cc. portion of the solution in each flask was transferred to an Evelyn tube previously rinsed with the solution, and the tubes were read in the Evelyn colorimeter with filter 440mμ. As checks, equivalent amounts of the snythetic triple salt solution were also diluted to 100 cc. and read at the same time.

Table 9

The efficiency of precipitation and washing.
Determination of known amounts of sodium chloride.

Sodium taken mgm.	Corrected galvanometer reading	Density	Sodium found mgm.	% error	K observed (from L&C)	K calc. (chart 5)	% Error
0	99^{2}	.0022	.0024				
0	99^{3}	.0011	.0012				
*.166	71^{2}	.1457			.867	.871	-.5
*.166	71^{2}	.1457			.867	.871	-.5
.168	71^{2}	.1457	.166	-1.2			
.168	71^{1}	.1472	.168	0			
.173	70^{1}	.1534	.175	+1.2			
*.231	62^{3}	.2024			.866	.860	+.7
*.231	62^{3}	.2024			.866	.860	+.7
.231	62^{3}	.2024	.231	0			
.231	63^{0}	.2007	.229	-.8			
.238	62^{0}	.2076	.237	- .4			
.238	62^{0}	.2076	.237	- .4			
*.301	55^{1}	.2577			.856	.851	+.5
*.301	55^{1}	.2577			.856	.851	+.5
.301	55^{1}	.2577	.301	0			
.301	55^{1}	.2577	.301	0			
.301	55^{2}	.2557	.299	- .8			
*.332	52^{1}	.2819			.848	.848	0
*.332	52^{1}	.2819			.848	.848	0

Table 9

The efficiency of precipitation and washing.
Determination of known amounts of sodium chloride.

Sodium taken mgm.	Corrected galvanometer reading	Density	Sodium found mgm.	Sodium error (from Lac)	K ob- ved (from Lac)	K calc. (Lac)	% Error
0	90	.0022	.0034				
0	89	.0011	.0012				
.166	71	.1457			.867	.871	-.5
.168	71	.1457			.867	.871	-.5
.168	71	.1457	.166	-1.2			
.168	71	.1472	.168	0			
.173	70	.1534	.175	+1.2			
.231	62	.2024			.666	.880	+.7
.231	62	.2024			.666	.880	+.7
.231	62	.2024	.231	0			
.231	62	.2007	.229	-.8			
.229	62	.2016	.227	-.4			
.229	62	.2016	.227	-.4			
.201	56	.1777			.886	.891	+.5
.201	56	.1777			.890	.891	+.5
.201	56	.1777	.201	0			
.201	56	.1777	.201	0			
.201	56	.1767	.198	-.8			
.282	82	.2519			.849	.846	0
.282	82	.2519			.849	.846	0

Table 9 (Cont.)

The efficiency of precipitation and washing.
Determination of known amounts of sodium chloride

Sodium taken mgm.	Corrected galvanometer reading	Density	Sodium found mgm.	% Error	K observed (from L&C)	K calc. (chart 5)	% Error
.332	52^{0}	.2840	.334	+.8			
.332	52^{1}	.2819	.332	0			
*.336	51^{3}	.2861			.852	.847	+.6
*.336	51^{3}	.2861			.852	.847	+.6
.336	52^{0}	.2840	.334	-.7			
.336	51^{3}	.2861	.336	0			
.336	52^{0}	.2840	.334	-.7			
*.399	46^{1}	.335	.399		.839	.840	-.1
*.399	46^{1}	.335	.399		.839	.840	-.1
.399	46^{1}	.335	.399				
.399	46^{0}	.337	.401	+.6			
.399	46^{1}	.335	.399	0			
*.463	41^{0}	.387			.838	.833	+.6
.463	41^{0}	.387	.463	0			
.463	41^{0}	.387	.463	0			
.463	41^{0}	.387	.463	0			
*.534	36^{0}	.444			.832	.825	+.8
*.534	36^{0}	.444			.832	.825	+.8
.534	36^{0}	.444	.534	0			
.534	36^{0}	.444	.534	0			

* Not precipitated or washed. Prepared from standard uranyl zinc sodium acetate solution.

Table 9 shows the results of these tests. It can be seen that the error in precipitation and washing averaged less than 1% within the range of .168 mgm.to .534 mgm. The last column of table 9 serves as a further check on the reproducibility of color of the triple salt with sulfosalicylic acid. No attempt was made in these experiments to study the precipitation and the washing separately, since the color produced by the precipitate can not be measured unless the excess of uranium is removed. These experiments show only that the errors in precipitation are offset by the errors in washing. However, the smallness of the error over the wide range would indicate that the error in either process is negligible .

Barber and Kolthoff (7) found that potassium, phosphate, and arsenates interfere in the gravimetric determination of sodium as uranyl zinc sodium acetate. In biological material the interference of arsenate may be ignored. Since potassium interferes only when it is present to the extent of 50 mgms. per cc. (7), it may be neglected in most biological fluids; however, since the concentration of potassium is much higher in cellular substance than in the surrounding fluids, it has been found advisable (67) to remove it by precipitation as perchlorate when cellular materials are analyzed. Since the present study is not concerned with such substances, the presence of potassium was ignored.

In the analysis of blood serum, the small concentration of phosphate can be ignored. In urine, however,

there is enough phosphate present to cause an appreciable precipitate. Butler and Tuthill (12), in applying the gravimetric method of Barber and Kolthoff (6), used solid calcium hydroxide for precipitating phosphate in preference to magnesia mixture, as recommended by the original workers (7). Overman and Garrett (69) used zinc carbonate. Hoffmann and Osgood (36), in developing a colorimetric method for sodium in urine, precipitated the phosphate along with the sodium, carried the insoluble uranyl phosphate through the washing process, and finally separated it by centrifuging after dissolving the sodium precipitate in water. Since these workers measured the color of the triple salt itself, which exhibits optimal color intensity when the precipitate from 0.1 cc. of urine is dissolved in 10 cc. or so of water, their procedure was very simple, consisting only of dissolving the precipitate in a measured volume of water, centrifuging down the insoluble uranyl phosphate, and reading the color of the supernatant fluid.

A modification of this method was used in the analyses of phosphate - containing solutions in the present study. Since the color developed from the triple salt with sulfosalicylic acid required dilution to 100 cc. to obtain accuracy of reading, it was necessary to measure an aliquot part of the phosphate-free supernatant solution for color development and measurement. The validity of this procedure was tested in the experiment described below and

summarized in table 10.

A solution containing 1.0000 gm. of pure anhydrous Na_2HPO_4 per liter was prepared. This solution corresponded to a 1:10 dilution of urine containing twice the average concentration of phosphate; 1 L. contained .4999gm. P_2O_5 and 1 cc. contained .3239 mgm. of sodium. From one portion of this solution the phosphate was removed by the method of Butler and Tuthill (12); 18 cc. of solution was shaken with .06 gms. solid $Ca(OH)_2$ for $\frac{1}{2}$ hour and filtered. A blank was prepared by shaking 18 cc. of water with .06 gms. $Ca(OH)_2$. The solutions were filtered and 1 cc. portions were delivered into centrifuge tubes from a calibrated microburette. Samples of distilled water and of untreated sodium phosphate solution were also analyzed by the same procedure. The precipitation and washing were carried out in the same manner as with the sodium chloride solutions, except that in the washing the precipitates were agitated by stirring with a glass rod. This was found to be necessary because the bulky phosphate precipitate tended to pack down and occlude some of the reagent, as evidenced by high results. After the final ether washing, the precipitates were dried for five minutes and shaken vigorously with 10 cc. of water to dissolve the triple salt. After centrifuging for 10 minutes, 5 cc. of the clear supernatant fluids were transferred to 50 cc. volumetric flasks. The flasks were filled about two thirds full with water, 2 cc.

Table 10

The non-interference of phosphates. Determination of sodium in disodium phosphate.

Sodium taken mgm.	Phosphate removed	Corrected galvanometer reading.	Density	Concentration of sodium C =L/K	Sodium found, mgm C-Blank	% Error
0	yes	99^{9}	.0033	.004		
0	yes	99^{1}	.0033	.004		
.326	yes	52^{2}	.2798	.330	.326	0
.326	yes	52^{2}	.2798	.330	.326	0
.326	yes	52^{3}	.2777	.328	.324	-.7
.326	yes	52^{1}	.2819	.332	.328	+.7
0	no	99^{1}	.0033	.004		
0	no	99^{1}	.0033	.004		
.326	no	52^{2}	.2798	.330	.326	0
.326	no	52^{1}	.2819	.332	.328	+.7
.326	no	52^{3}	.2777	.328	.324	-.7
.326	no	52^{2}	.2798	.330	.326	0

of sulfosalicylic acid and 2 cc. of sodium acetate were added to each, and 10-15 cc. of the solution read in the colorimeter as before. As shown in table 10, there was no difference in reliability between results obtained with and without removal of phosphates by precipitation with calcium hydroxide.

Since slightly acidified uranyl salts have been shown (34) to be excellent protein precipitants, it is necessary to remove or to destroy the protein in biological fluids before proceding with the determination of sodium. The removal of protein by precipitation is far less time-consuming than is the destruction of protein by ashing, but the results of mineral determinations on deproteinized samples are not as reliable as those on ashed samples. The determinations of minerals in trichloracetic acid filtrates have been studied extensively by Van Slyke, Hiller, and Berthelsen (95), Hald (35), Oberst (67), Grigant and Boutroux (34), and Ball and Sadusk (4). All these authors found that values obtained from trichloracetic acid filtrates were from 2 to 5% higher than those from ashed samples, and all explained the error as due to volume changes caused by precipitating the protein. Some confirmation of this view is found in the results of Oberst (67), who found discrepancies of more than 5% between ashed and precipitated samples of cellular material, in which the protein content is higher than in plasma.

In urines, which contain little or no protein, precipitation by mercuric chloride (12) or by trichloracetic acid (82)

has not been found to introduce serious error. In the present study, 1:10 trichloracetic acid filtrates of urine were made routinely. Details of the procedure and results will be shown in the next section.

When dealing with blood serum in the present study, determinations were made both on samples ashed by the procedure of Hoffman and Osgood (36) and on 1:10 trichloracetic acid filtrates. Details of procedure and results are shown in the section dealing with the adaptation of the method to blood serum. Table 11 shows that values with trichloracetic acid filtrates were from 0.9 to 4.0% higher than those on ashed samples of the same blood.

Application of method to biological fluids.

The theoretical, historical, and experimental basis for all the steps involved in the application of the method to blood, urine, and cerebrospinal fluid have been discussed in the preceding sections. The assembled procedures are presented here.

Reagents used for sodium determinations in trichloracetic acid filtrates of biological materials are:

1. Trichloracetic acid, 20% solution.
2. Ashless filter paper, Whatman No. 40.
3. Uranyl zinc acetate reagent, prepared according to Weinbach (96). Solution A: 77 gm. of uranyl acetate $UO_2(CH_3COO)_2 \cdot 2H_2O$, and 14 cc. of glacial acetic acid are dissolved by heating and stirring in 400 cc. of water. Solution B: 231 gm. of zinc acetate, $Zn(CH_3COO)_2 \cdot 2H_2O$, and 7 cc. of

glacial acetic acid are dissolved by heating and stirring in 400 cc. of water. The two solutions are mixed and diluted to 1 liter while still warm. After standing 24 hours, the mixed solution is ready for use. It is filtered immediately before using.

4. Ethyl alcohol, 95%.

5. Ethyl acetate-acetic acid wash reagent: 300 cc. of C. P. ethyl acetate diluted to 1 liter with glacial acetic acid.

6. Ethyl ether, redistilled.

7. Sulfosalicylic acid, 5% solution.

8. Sodium acetate, 10% solution: 50 gm. of C. P. sodium acetate, $NaCH_3COO.\ 3H_2O$ dissolved in water and diluted to 500 cc.

Other reagents, used only in sodium determinations in ashed samples, are:

1. Sulfuric acid, 6N solution.

2. Nitric acid, concentrated.

3. Hydrogen peroxide, 30%.

For the determination of sodium in blood serum, urine or cerebrospinal fluid, 1 cc. of 1:10 trichloracetic acid filtrate of the material or 0.1 cc. of material wet-washed by the procedure of Hoffman and Osgood (36) are analyzed.

In the preparation of trichloracetic acid filtrates, 1 cc. of biological material is delivered from a pipette (calibrated

to deliver the material measured) into 7 cc. of distilled water in a test tube. While shaking the tube gently, 2 cc. of 20% trichloracetic acid is added. The contents of the tube are thoroughly mixed by stirring and allowed to stand for ten minutes. After filtering through an ashless filter paper, 1.0 cc. of the clear filtrate is delivered from a pipette calibrated for delivery of water into a 15 cc. conical centrifuge tube for treatment with uranyl zinc acetate reagent.

In the preparation of ashed samples, 0.1 cc. of the material to be analyzed is delivered from a pipette calibrated to deliver that material into a 15 cc. conical centrifuge tube of heat-resistant glass. After adding .2 cc. of 6N sulfuric acid and .1 cc. of concentrated nitric acid, the tube is placed in a beaker of boiling water for 10 minutes or longer. The tube is then removed from the water bath and the contents charred over a free flame. By allowing a small flame from the microburner to strike just above the level of the liquid in the constantly shaken tube, very little danger of loss from spattering is encountered. The tube is allowed to cool while another is being similarly treated. Then is added one drop of 30% H_2O_2 from a capillary pipette. The contents are again evaporated over a free flame until SO_3 fumes fill the tube, and allowed to cool during the treatment of the second tube. The addition of hydrogen peroxide is continued until, after evaporation to SO_3 fumes, only a colorless drop of liquid remains in the tube. (All tubes in any series are treated with equal

amounts of H_2O_2 in order to insure constancy of the blank; 6 drops or less will generally be enough to produce a clear solution). After the final evaporation with H_2O_2, the tube is allowed to cool for 10 minutes or more, and to it are added 0.9 cc. of water. After mixing the contents of the tube, it is ready for treatment with uranyl acetate reagent.

To 1 cc. of solution prepared by one of the methods described above, 5 cc. of freshly filtered uranyl zinc acetate reagent is added. At 5 minute intervals are added seven 0.3 cc. portions of ethyl alcohol. These additions must occupy at least ½ hour, and may take longer. After each of the first five additions of alcohol the liquid in the tube is mixed, first by tapping the bottom of the tube, producing a rotatory motion in the upper part of the liquid, then by rolling the tube back and forth between the palms of the hands, thus producing effective mixing in the lowest part of the tube. The last two additions of alcohol serve to wash down the walls of the tube. They are allowed to remain layered on the solution. After the last addition of alcohol, the tube is centrifuged at 2000 r.p.m. for ten minutes, decanted, inverted, and allowed to drain for five minutes. The mouth of the tube is wiped dry and the precipitate is agitated by blowing on it a fine stream of about 2 cc. of ethyl acetate-acetic acid wash liquid.* The walls of

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* In the case of urines which contain much phosphate the precipitate must be agitated by stirring with a glass rod, which is washed off with the wash liquid after each use.

the tube are washed down with a small amount of the liquid. Centrifuging for ten minutes, draining for five minutes, and wiping are carried out as before, and about 5 cc. of ether are used to wash the precipitate and the walls of the tube; precipitates from urines must be stirred with a glass rod. The tube is centrifuged for 5 minutes, decanted, and drained for 1 minute. (Longer draining may allow the flaky precipitate to become so dry that it crumbles and falls out of the tube). The precipitate is washed a second time with 5 cc. of ether, centrifuged 5 minutes, decanted, and drained for one minute. The tube is placed in a warm place for 5 minutes to evaporate the last traces of ether. To this point the procedure is identical for blood, urine, and cerebrospinal fluids, with the exception of the agitation of precipitates, as noted.

For blood and cerebrospinal fluid, the remainder of the procedure is identical: the washed, dried precipitate is dissolved in 4-5 cc. of water and transferred quantitatively to a 100 cc. volumetric flask. The solution in the flask is diluted to about 70 cc., and to it are added in order 4 cc. of 5% sulfosalicylic acid, 4 cc. of 10% sodium acetate, and water to make 100 cc. The contents of the flask are mixed thoroughly and a 15 cc. portion transferred to an Evelyn colorimeter tube and read in the colorimeter, using the 440 mμ filter. The instrument is set to read 100 with a tube containing 4 cc. of each of 5% sulfosalicylic acid and 10% sodium acetate in 100 cc. The L value corresponding to

the corrected galvanometer reading is read from the chart supplied with the colorimeter, and the concentration of sodium in the original material is calculated from the concentration in the sample tube (read from chart 7) by subtracting the sodium content of a similarly treated blank.*

If c is the concentration of sodium in the sample tube, b is the concentration of sodium in the blank tube (obtained by dividing L by the correct value of K from chart 5), and v is the volume of serum or spinal fluid taken in the ashed sample or trichloracetic acid filtrate, then

$$\frac{100(c-b)}{v} = \text{mgms. \% of sodium in serum or spinal fluid.}$$

The precipitate from urine is shaken with 10 cc. of water; the triple salt dissolves while the uranyl phosphate remains behind as a gelatinous precipitate. The tube is centrifuged for ten minutes at 200 r.p.m. Five cubic centimeters of the clear supernatant fluid is pipetted into a 50 cc. volumetric flask and diluted to about 35 cc. with water. Then are added in order 2 cc. of 5% sulfosalicylic acid, 2 cc. of 10% sodium acetate, and water to make 50 cc. The solution is read in the colorimeter in the same manner as is a solution of blood precipitate. Since the colored solution contained

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* The reagent blank for trichloracetic acid filtrates is prepared by diluting two parts of 20% trichloracetic acid with 8 parts of water, filtering, adding 1 cc. to a centrifuged tube, and carrying out the precipitation and washing as with blood filtrates. The blank for ashed samples is prepared by treating 0.1 cc. of water with sulfuric acid, nitric acid, and hydrogen peroxide, and carrying out the procedure exactly as with blood serum.

half the precipitate from the sample in 50 cc. of final volume, the concentration of color is the same as if all the precipitate was dissolved in 100cc., so the same charts can be used for finding the concentrations of sodium in sample and blank, and the calculation of results is the same as for blood or cerebrospinal fluid.

Table 11 shows the results of analyses of fifteen samples of blood. Ashed samples and trichloracetic acid filtrates of each blood were prepared and were analyzed by the procedure given above. The average value of the sodium concentration in 15 normal blood sera, determined on ashed samples, was found to be 334 mgms. per 100 cc. The range was from 324 to 342 mgms. per 100 cc. As determined on trichloracetic acid filtrates, the average was 341 mgms. per 100cc. and the range from 333 to 349 mgms. per 100 cc. The average value for ashed samples is in excellent agreement with the figure of 335 mgms. per 100cc., which Peters and Van Slyke (71) give as the normal concentration. The variation of the extremes was less than ± 4%, which Hoffman and Osgood (36) state as the normal variation of human blood sodium.

Trichloracetic acid filtrates gave values from .9 to 4.0% higher than ashed samples of the same blood. The average difference was 2.3%. This figure compares favorably with those reported by Van Slyke, Hiller and Berthelsen (95), Hald (35), Grigant and Boutroux (34), and Ball and Sadusk (4).

Table 12 shows the results of the analyses of ten samples

Table 11

Determination of sodium in blood sera.

No.	Blood taken cc.	Treatment *	L**	Total sodium found mgms.	Sodium in blood, mgms. (total -blank)	Sodium in blood mgms. %	% diff. A & T
0	0	A	.0088	.020			
	0	T	.0066	.014			
1	.200	A	.2924	.693	.673	337.	
	.202	T	.297	.702	.688	341.	1.2
	.202	T	.297	.702	.688	341.	1.2
2	.200	A	.2882	.682	.662	331.	
	.200	A	.2882	.682	.662	331.	
	.202	T	.2903	.688	.674	334.	.9
	.202	T	.2903	.688	.674	334.	.9
3	.200	A	.2882	.682	.662	331.	
	.202	T	.297	.704	.690	342.	3.3
	.202	T	.297	.704	.690	342.	3.3
4	.200	A	.2903	.688	.668	334.	
	.200	A	.2882	.682	.662	331.	
	.202	T	.299	.710	.696	345.	3.8
	.202	T	.297	.704	.690	342.	2.8
5	.200	A	.2924	.693	.673	337.	
	.202	T	.297	.704	.690	342.	1.5
	.202	T	.297	.704	.690	342.	1.5
6	.200	A	.297	.704	.684	342.	
	.200	A	.297	.704	.684	342.	
	.202	T	.305	.720	.706	349.	2.0
	.202	T	.305	.720	.706	349.	2.0
7	.200	A	.295	.700	.680	340.	
	.202	T	.299	.710	.696	345.	1.5
8	.200	A	.2924	.693	.673	337.	
	.200	A	.2924	.693	.673	337.	
	.202	T	.299	.710	.696	345.	2.4
	.202	T	.299	.710	.696	345.	2.4

* A = ashed. T = trichloracetic acid.
** Final volume of colored solution was 200 cc.

Table 11 (cont.)

Determination of sodium in blood sera.

No.	Blood taken cc.	Treatment *	L**	Total sodium found, mgms.	Sodium in blood, (total-blank)	Sodium in blood, mgms.	%	% diff. A & T
00	0	A	.0044	.005				
	0	T	.0144	.016				
9	.103	A	.2882	.341	.336	326.		
	.103	A	.2861	.339	.334	324.		
	.101	T	.299	.355	.339	336.		3.4
	.101	T	.301	.357	.341	338.		4.0
10	.103	A	.2882	.341	.336	326.		
	.103	A	.2882	.341	.336	326.		
	.101	T	.301	.357	.341	338.		3.7
	.101	T	.301	.357	.341	338.		3.7
11	.103	A	.2924	.347	.342	332.		
	.103	A	.2924	.347	.342	332.		
	.101	T	.305	.363	.347	344.		3.6
	.101	T	.305	.363	.347	344.		3.6
12	.103	A	.295	.350	.345	335.		
	.103	A	.295	.350	.345	335.		
	.101	T	.305	.363	.347	344.		2.8
13	.103	A	.2903	.344	.339	329.		
	.103	A	.2882	.341	.336	326.		
	.101	T	.297	.352	.336	333.		1.7
	.101	T	.297	.352	.336	333.		1.7
000	0	A	.0166	.019				
	0	T	.0121	.014				
14	.097	A	.2903	.344	.325	335.		
	.101	T	.299	.355	.341	338.		.9
15	.097	A	.2903	.344	.325	335.		
	.097	A	.2903	.344	.325	335.		
	.101	T	.301	.358	.344	341.		1.8
	.101	T	.299	.355	.341	338.		.9

* A ashed. T = trichloracetic acid.

** Final volume of colored solution was 100cc.

Table 12

Determination of sodium in urines

No.	Urine taken, cc.	Treatment *	L	Total sodium found, mgms.	Sodium in urine, mgms.	Sodium in urine, mgms. %
0	0	A	.0044	.005		
	0	T	.0144	.016		
1	.103	A	.328	.389	.384	374
	.103	A	.332	.394	.389	378
	.101	T	.342	.407	.391	387
	.101	T	.342	.407	.391	387
2	.103	A	.409	.493	.488	475
	.103	A	.406	.489	.484	471
	.101	T	.426	.514	.498	493
	.101	T	.423	.511	.495	490
3	.101	T	.349	.416	.400	396
4	.101	T	.1565	.180	.164	162
	.101	T	.1565	.180	.164	162
5	.101	T	.1192	.137	.121	120
	.101	T	.1192	.137	.121	120
6	.101	T	.1805	.208	.192	190
	.101	T	.1821	.210	.194	192
7	.101	T	.1905	.221	.205	203
	.101	T	.1888	.219	.203	201
8	.101	T	.2007	.233	.217	215
	.101	T	.1973	.230	.214	212
9	.101	T	.1739	.202	.188	186
	.101	T	.1739	.202	.188	186
10	.101	T	.1805	.208	.192	190
	.101	T	.1821	.210	.194	190

* A = ashed. T = trichloracetic acid.

Table 12

Determination of sodium in urines.

No.	Urine taken, cc.	Treatment *	I	Total sodium found, mgms.	Sodium in urine, mgms.	Sodium in urine, mgms. %
0	0	A	.0014	.005		
	0	T	.0144	.018		
1	103	A	.328	.388	.384	274
	103	A	.332	.394	.389	276
	101	T	.342	.407	.391	387
	101	T	.342	.407	.391	387
2	103	A	.409	.483	.469	473
	103	A	.405	.480	.484	471
	101	T	.426	.514	.498	492
	101	T	.425	.511	.495	490
3	101	T	.349	.410	.400	398
4	101	T	.1565	.180	.164	162
	101	T	.1565	.180	.164	162
5	101	T	.1382	.157	.121	120
	101	T	.1382	.157	.121	120
6	101	T	.1808	.208	.162	160
	101	T	.1821	.210	.164	162
7	101	T	.1800	.221	.208	203
	101	T	.1886	.219	.203	201
8	101	T	.2007	.232	.217	215
	101	T	.1973	.230	.214	212
9	101	T	.1732	.202	.186	184
	101	T	.1739	.202	.186	184
10	101	T	.1805	.208	.192	190
	101	T	.1821	.210	.194	192

* A = saline. T = trichloracetic acid.

of urine. Excellent agreement was found between duplicate determinations on both trichloracetic acid filtrates and ashed samples, but the determinations on trichloracetic acid filtrates, performed on specimens 1 and 2 gave results about 4% higher than those on ashed samples. This discrepancy does not seem explainable on the basis of any protein present, as the precipitate obtained in both cases was negligible. The agreement of duplicates in the range of 120 to 493 mgms. % of sodium per 100 cc. indicates that the method is applicable to samples of urine with sodium concentrations in this range.

Two samples of cerebrospinal fluid were analyzed, both by ashed samples and trichloracetic acid filtrates. The results, tabulated in table 13, likewise show higher values (average 2.3%) for trichloracetic acid filtrates than for ashed samples. The actual values are comparable with those of blood sera.

The accuracy of the method as applied to biological fluids is demonstrated by the agreement between duplicate samples treated in the same way. As an absolute check on the accuracy, known amounts of sodium chloride were carried through the entire procedure, including ashing and preparing the filtrate. Sodium was recovered from ashed samples with errors of -.3% and +.6% from trichloracetic acid filtrates with errors of 0.0 and +.6% as shown in table 14.

Four experiments were performed in which known amounts of sodium chloride and of analyzed blood were ashed and carried through the procedure. Sodium was recovered with errors of 0.0, +.3, and +.3%. Results are shown in table 14.

Table 13

Determination of sodium in cerebrospinal fluids.

No.	C. S. F. taken, cc.	Treatment*	L	Total sodium found, mgms.	Sodium in C.S. F. mgms. (Total-blank)	Sodium in C. S. F. mgms. %
0	0	A	.0044	.005		
	0	A	.0144	.016		
1	.103	A	.295	.350	.345	335.
	.103	A	.295	.350	.345	335.
	.101	T	.303	.360	.344	341.
	.101	T	.303	.360	.344	341.
2	.103	A	.2924	.347	.342	332.
	.101	T	.303	.360	.344	341.
	.101	T	.301	.357	.341	338.

*A = ashed. T= trichloracetic acid.

Table 13

Determination of sodium in cerebrospinal fluids.

No.	C. S. F. taken, cc.	Treatment	I.	Total sodium found, mgms.	Sodium in C. S. F. mgms. (Total-blank)	Sodium in C. S. F. %
0	0		A	.0044	.003	
	0		A	.0144	.013	
1	.103		A	.380	.340	.330
	.103		A	.380	.340	.330
	.101		T	.360	.344	.341
	.101		T	.360	.344	.341
2	.103		A	.3474	.342	.332
	.101		T	.360	.314	.341
	.101		T	.361	.341	.338

A = saline. T = trichloroacetic acid.

Table 14

Recovery of sodium from mixtures of analyzed blood and standard sodium chloride solution

Treatment*	Sodium taken, mgm. from NaCl	Sodium taken, mgm. from blood	Volume final soln., cc.	L	Total sodium found, mgm.	Sodium from NaCl= total-blood-blank	% error
A	0	0	100	.0044	.005		
A	.340	0	100	.2903	.344	.339	-.3
A	.340	0	100	.2924	.347	.342	+.6
A	0	**	100	.2882	.341	.336) from	
A	0	**	100	.2882	.341	.336)blood	
A	.340	.336	200	.2882	.681	.340	0
A	.340	.336	200	.2882	.681	.340	0
A	0	**	100	.2924	.347	.342) from	
A	0	**	100	.2924	.347	.342)blood	
A	.340	.342	200	.2903	.688	.341	+.3
A	.340	.342	200	.2903	.688	.341	+.3
T	0	0	100	.0144	.016		
T	.334	0	100	.295	.350	.334	0
T	.334	0	100	.297	.352	.336	+.6

* A= ashed. T= trichloracetic acid.

** Same quantity (.103 cc.) of blood taken here as added in following two recovery experiments.

Summary

1. The limitations of existing methods for the determination of sodium in biological fluids have been discussed.
2. It has been shown that the Evelyn colorimeter does not detect differences in concentration of less than .6%.
3. The color developed from the uranyl ion with sulfosalicylic acid and sodium acetate has been studied. The conformity of this color with Beer's law has been studied, and the color has been shown to be reproducible and to be stable with respect to temperature and time.
4. A new method, consisting of precipitation of uranyl zinc sodium acetate from alcoholic solution, removal of excess precipitant by washing with ethyl acetate in acetic acid and with ether, removal of phosphate as uranyl phosphate, and photoelectric measurement of the color developed with sulfosalicylic acid and sodium acetate, has been developed for the determination of sodium in biological fluids.
5. Recovery of sodium from known amounts of sodium chloride, both in simple aqueous solution and added to biological fluids, has indicated that the various steps, as well as the assembled procedure, have a maximum error of less than 1%.

Abstract

A calculation of the percentage of error involved in the use of the Evelyn photoelectric colorimeter has shown that since the percentage of light transmitted by a colored solution can be measured to within one-quarter of one percent and since the light transmitted is a logarithmic function of the concentration of absorbing material, the error can be held to a maximum of .6 - .7% by the proper choice of sizes of samples. This accuracy, together with the saving of time effected by photoelectric colorimetry as compared with titrations or weighings, creates a definite demand for a photoelectric method for the determination of sodium. Since the normal variation in concentration of sodium in human blood serum ranges only four percent in either direction from the mean value, a method for the clinical determination of sodium must have an error not greater than one percent.

A review of the literature reveals only one photoelectric method for the determination of sodium. This method, published by Hoffman and Osgood, is based on the precipitation of sodium as uranyl zinc sodium acetate, the solution of the precipitate, and the photoelectric measurement of the yellow color of the uranyl ion. The authors found that the color is relatively unstable to heat and to acidity.

Preliminary experiments on the colors imparted by phenolic

acids to uranyl salts showed that sulfosalicylic acid, a common, colorless, very soluble reagent, imparts to uranyl salts an orange color exhibiting a narrow absorption band with maximum absorption at 455 millimicrons. Further investigation showed that the color can be intensified by the addition of sodium acetate. The color conforms with Beer's law to well within one percent over ranges of concentration of uranium of ten percent. Over a wide range of concentration the deviation from Beer's law is apparently directly proportional to the concentration of uranium, so that a calculation of concentration from color density is much simplified. The color is reproducible, stable over a period of three hours, and is not markedly affected by changes of temperature. The color is somewhat unstable to changes of acidity. Careful standardization of the color reagents eliminated any errors from this source.

All methods so far proposed for the washing of the precipitated uranyl zinc sodium acetate have called for the use of liquids saturated with the triple salt. Hoffman and Osgood showed that these wash reagents are objectionable because some of the triple salt is precipitated from them when they are brought in contact with uranyl zinc acetate. The use of the precipitation method of Weinbach and of a new wash reagent

containing ethyl acetate in acetic acid eliminated this excessive precipitation and afforded quantitative recovery at room temperature of sodium in amounts ranging from 0.165 to 0.534 milligrams.

Since uranyl salts precipitate phosphate as an extremely insoluble uranyl phosphate, previous workers have removed phosphate from urines, where its concentration may be high, before precipitating the sodium. The phosphate content of blood and cerebrospinal fluid is insufficient to interfere with the determination of sodium. In the analysis of urines in the present study phosphate was precipitated along with the sodium by the uranium, and was carried through the washing process with the sodium compound. It was finally removed by dissolving the triple salt in a measured quantity of water, centrifuging off the insoluble uranyl phosphate, and taking an aliquot part of the clear supernatant fluid for completion of the analysis. This procedure was modified from that of Hoffman and Osgood. Determinations of sodium in solutions of disodium phosphate having a concentration of phosphate twice that encountered in urine indicated that this procedure gives results as reliable as those given by procedures in which phosphate is removed, provided that care be taken, in washing the precipitate, to remove any occluded uranium.

The procedure for the determination of sodium is

as follows: the sample is prepared for analysis by the removal of protein either by wet ashing or by treatment with trichloracetic acid. In the wet ashing process of Hoffman and Osgood, used in these experiments, 0.1 cc. of the material is treated in a 15 cc. conical centrifuge tube of heat-resistant glass with .2 cc. of 6 N sulfric acid and .1 cc. of concentrated nitric acid and, after evaporating the solution to SO_3 fumes before each addition, 6 drops of 30% H_2O_2, added one drop at a time. After the final addition of H_2O_2 the solution is evaporated to SO_3 fumes, cooled, and diluted with .9 cc. of water. Trichloracetic acid filtrates are prepared by mixing 1.0 cc. of biological material with 7.0 cc. of water and 2.0 cc. of 20% trichloracetic acid. After standing for 10 minutes the solution is filtered through an ashless filter paper. One cubic centimeter of the filtrate is transferred to a 15 cc. centrifuge tube for analysis. According to the method used above, 0.1 cc. or 1.0 cc. of water is treated in exactly the same way for the reagent blank.

The precipitation is accomplished by adding to the 1 cc. sample (equivalent to 0.1 cc. of biological material) 5 cc. of Weinbach's uranyl zinc acetate reagent and seven 0.3 cc. portions of 95% ethyl alcohol. The additions of alcohol are spread over one-half hour or more, and the alcohol is thoroughly mixed with the liquid in the tube after the first five additions; the last two portions of alcohol serve to wash

down the walls of the tube. The tube is centrifuged for 10 minutes at 2000 r.p.m., the supernetant fluid decanted, and the tube inverted and allowed to drain for 5 minutes. The precipitate is washed by blowing onto it about 2 cc. of 30 % ethyl acetate in acetic acid. After agitating the precipitate in the wash liquid, the walls of the tube are washed down with a small portion of the liquid. The tube is again centrifuged for 10 minutes and drained for 5 minutes. Washing with 5 cc. of ether, centrifuging 5 minutes, and draining 1 minute are carried out twice. The tube is placed upright in a warm place for 5 minutes to remove the last traces of ether. If the phosphate content of the original material is negligible, the precipitate is dissolved in water, transferred quantitatively to a 100 cc. volumetric flask, diluted to about 70 cc. with water, mixed with 4 cc. each of 5 % sulfosalicylic acid and of 10 % sodium acetate, and diluted to the mark. Precipitates from phosphate-containing fluids are shaken with 10 cc. of water, which dissolves the triple salt. The insoluble uranyl phosphate is centrifuged off and 5 cc. of the supernatant fluid is transferred to a 50 cc. volumetric flask, treated with half quantities of the color reagents, and diluted to the mark. A color blank is prepared by diluting 4 cc. of each of the color reagents to 100 cc. with water. Portions of 15 cc. from each of the flasks — biological fluid, reagent blank, and color blank — are transferred to Evelyn colorimeter tubes and read in the Evelyn

photoelectric colorimeter, using a blue filter showing maximum transmission at 440 millimicrons. The galvanometer is set to read 100 with the color blank, and readings are taken on the other tubes. From the galvanometer readings the color densities of the tubes are calculated. The sodium concentration in each tube is calculated from the experimentally determined relationship of concentration to density. Where L is the "optical density" (2-log G) and C is the concentration of sodium in milligrams per 100 cc. of colored solution, L/C= .848 in the ranges encountered in the analyses of blood. By subtracting the concentration of sodium in the reagent blank from that in the sample tube and dividing by the volume of biological fluid taken for analysis, the amount of sodium in 1 cc. of fluid is obtained.

The sodium concentration in 15 normal human blood sera, determined on ashed samples, was found to vary between 324 and 342 (average 334) mgms. per 100cc. Values obtained on trichloracetic acid filtrates averaged 2.3% higher. Concentrations observed on 2 samples of cerebrospinal fluid were comparable to those observed in blood sera. Samples of ten urines varied in sodium content between 162 and 493 milligrams per 100 cc.

Analyses of known amounts of sodium chloride, both in simple aqueous solution and mixed with measured amounts of analyzed blood serum, indicated that the error involved in the use of the method varies from -.3% to +.6%.

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